

## Screening Antiproliferative and Antimicrobial Effects of Ethyl Acetate Extract Driven from *Camellia sinensis* Flowers

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Received December 30, 2019; Accepted January 06, 2020; Published March 28, 2020

### ABSTRACT

Plants have been used to prevent or to treat some diseases for thousand of years. As resistance to antibiotic and cancer drugs has been increasing rapidly in recent years, the scientific world has turned its focus on plants used in traditional medicine to counteract this trend. Studies conducted with the leaves of *Camellia sinensis* have reported that some components in the contents of the tea plant may have varying degrees of antimicrobial and anti-cancer activities.

The antiproliferative effects of ethyl acetate (EtOAc) extract obtained from the flowers against non-transformed and transformed cells were evaluated with the MTT (3-[4,5-Dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide) assay. Additionally, the extract's antimicrobial activity against some gram- positive (Gr+), Gram-negative (Gr-) bacteria and *Candida* spp. was investigated by an agar well diffusion assay and minimum inhibition concentration (MIC) was carried out using the broth microdilution method.

The EtOAc extract at 200 µg/ml concentration exhibited growth inhibitory effects on all cancer cells, but had no effect on non-transformed cells. At this concentration, the inhibitory effect on cancer cells was statistically significant ( $p < 0.05$ ) in comparison to non-transformed ARPE-19 and HEL299 cell lines. However, the anti-proliferative effect at 100 µg/ml was retained against endometrial cancer cell line (CRL-2923) only. Notably, CRL-2923 cell line was found to be susceptible to the extract.

The extract produced inhibition zones ranging from 10.0 mm to 17.3 mm for the bacteria and from 19.0 to 21.33 for yeast. Two Gr(+) and two Gr(-) bacteria were susceptible to the extract, while the other microorganisms that were used in the study were not affected. In particular, the most susceptible Gr (+) and Gr (-) bacteria were *Staphylococcus aureus* and *Klebsiella pneumoniae* with MIC values of 62.25 µg/ml and 500 µg/ml, respectively. Additionally, the extract exhibited a good anti-fungi activity against both *Candida* species with the same MIC value.

**Keywords:** Cancer, Antimicrobial, *Camellia sinensis*, ARPE-19, HEL299 A549, CRL-2923, HeLa

### INTRODUCTION

Over 60% of the drugs used in cancer treatment [1] and 65% of antibiotics are natural products or plant derivatives [2]. Tea (*Camellia sinensis*), a member of the Theaceae family, is a short, green-leaved plant that grows in humid climates. The plant, which is native to China, has been introduced into the world over time [3] and widely grows in the province of Rize in Turkey. Historical sources show that the first use of tea dates back to 2700 BC [3,4]. At present, tea is the most commonly consumed beverage after water, in the forms of black, green and white tea.

In recent years, the health benefit of tea has been understood. The antioxidant, anti-cancer and antimicrobial properties of bioactive substances obtained from the tea

leaves have been the subject of numerous scientific studies [5]. The chemical composition of tea is well documented. The majority consists of polyphenols, alkaloids, essential oils and polysaccharides. However, changes in chemical

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**Citation:** Eksi S, Ejder N, Ureyen UZ, Yazici Z, Uzunok B, et al. (2020) Screening Antiproliferative and Antimicrobial Effects of Ethyl Acetate Extract Driven from *Camellia sinensis* Flowers. J Pharm Drug Res, 3(2): 319-328.

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composition occur during the developmental stages of the plant [6, 7]. Numerous studies have emphasized that polyphenols and polysaccharides may induce antioxidants, anti-inflammatory, antiallergic [3,8] antimicrobial [9,10] and anti-cancer [11-17] activities.

It is evident from the relevant literature that most scientific studies with tea focused on the compounds obtained from the leaves. More recent studies, however, incorporated tea flowers that have been shown to have unique compounds/properties [6]. Additionally, it was observed that tea flowers are also rich in polyphenols, alkaloids, essential oils and polysaccharides [18,19]. As the extraction method and substance can affect the amount and nature of the contents, we set out to investigate the ethyl acetate extracts of tea flowers for their antiproliferative and antimicrobial activities against transformed and non-transformed human cell lines.

## MATERIALS AND METHODS

### Chemicals

DMSO (dimethyl sulfoxide), MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Taxol, D-PBS (Phosphate buffered saline) and trypan blue (0.4%), ampicillin, gentamicin, ciprofloxacin and amphotericin-B, ethyl acetate (EtOAc) were purchased from Sigma-Aldrich. RPMI-1640 (Hyclone), fetal bovine serum (FBS), antibiotics (100 µg/ml streptomycin+100 U/ml penicillin), 0.25% trypsin-EDTA and Dulbecco's modified Eagle medium (DMEM) were obtained from GIBCO. Brain heart Infusion Broth (BHIB), Brain heart Infusion Agar (BHIA), Mueller Hinton II Broth (MHB-II), Mueller Hinton agar (MHA) and methyl blue dye were purchased from MERCK.

### Collection and storage of the plant flowers

Tea flowers were collected from the town of İkizdere, Rize, in Turkey in October 2018. They were stored frozen in polyethylene bags at -20°C until extraction.

### Preparation of the flowers extract

15 g of frozen flowers was pulverized, suspended in 150 mL of EtOAc and extracted on a rotary shaker at room temperature for 48 h. The extract was filtered through Whatman paper and evaporated at 40°C by using a rotary evaporator (LabTech EV311). There after, the residue was resuspended in DMSO at 100 mg/ml and stored at -20°C for further use. Before final use, the desired test concentrations were prepared from the stocks in cell culture medium. The negative control received the corresponding amount of DMSO alone.

### Cell lines and cell culture

Adenocarcinomic human alveolar basal epithelial cell line A-549 and human cervical cancer epithelial cell line (HeLa) were kindly provided by Prof. Fikretin Sahin (Yeditepe University, Istanbul, Turkey), human endometrial

adenocarcinoma cell line CRL-2923 was a gift from Prof. Bedia Agachan Cakmaoglu (Istanbul University, Istanbul, Turkey) and diploid ARPE-19 retinal pigment epithelial cell line was kindly provided by Dr. Muradiye Acar (Turgut Ozal University, Ankara, Turkey). Human diploid lung fibroblasts HEL-299 were obtained from the American Type Culture Collection (ATCC).

The cell lines were maintained in RPMI-1640 or DMEM with 10% heat-inactivated FBS and antibiotics (100 µg/ml streptomycin+100 U/ml penicillin) in T25 culture flasks at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. At 70-80% confluency, the cells were passaged enzymatically with 0.25% trypsin and sub-cultured in T25 plastic flasks for further maintenance. The culture media were replaced every 2 days.

### Morphological studies

Morphological studies were performed as described earlier by Eksi et al. [20]. Briefly, the transformed Hela, A549, CRL-2923 cell lines ( $1 \times 10^4$  cells/ml) and non-transformed ARPE cells ( $2 \times 10^4$  cells/ml) were seeded into 24-well culture plates with 1000 µL of cell culture media. After overnight incubation, flower extract (300 µg/ml) or the corresponding DMSO (max. 0.4%) alone were added. Taxol (5 nM) was used as positive control. Subsequently, the cultures were cultivated at 37°C for 24 h in an atmosphere of 5% CO<sub>2</sub> and 95% air in an incubator. At the end of the treatment, cellular morphology was viewed under an inverted microscope at a magnification of 10x (Olympus CKX410). The morphological changes caused by the extract and the controls on the non-transformed and transformed cells were evaluated and photographed using a digital microscope camera (Olympus SC30).

### Determination of antiproliferative activity (MTT test)

Cytotoxic activity was evaluated based on the method described by Mosmann [21], with minor modifications. After trypsinization, for the transformed cells,  $1 \times 10^3$  cells/well were seeded into flat-bottom 96-well microtiter plates in 100 µl of growth medium in duplicates and allowed to adhere overnight. The cell number for the non-transformed diploid cells was adjusted to  $2 \times 10^3$  cells/well. The next day, the extract or controls (DMSO and Taxol) were added to the first wells of the microtiter plate in duplicates. Subsequently, serial 2x dilutions ranging from 200 µg/ml to 6.25 µg/ml were made in the plates, and all plates were incubated at 37°C in a 5% CO<sub>2</sub> incubator for 48 h. Then previously filtered 10 µl of MTT solution (5 mg/ml in water) was added to each well, and the cells were incubated for an additional 4 h at 37°C. Subsequently, the microplates were centrifuged at 500 rpm for 5 min (Hettich Zentrifugen Rotina 380A). Afterwards, the medium was removed and the formazan crystals that formed in the viable cells during the MTT treatment were dissolved by adding 100 µl of DMSO per well. The plates were incubated at

37°C for 20 more min to allow complete solubilization. At the end, absorbance was measured at 570 nm using an ELISA microplate reader (BioTek ELX800). All experiments were performed in duplicates and repeated at least three times. Growth inhibition was calculated using the following formula [22]:

$$\% \text{ growth inhibition} = \frac{[(\text{negative control OD} - \text{sample OD}) / \text{negative control OD}] \times 100}{}$$

### Microorganisms used for screening

A total of 13 human pathogenic microbial organisms were used to assess the antimicrobial properties of the extract. Mainly Gr (+) bacteria; *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Enterococcus fecalis* (ATCC 29212) and Gr (-) bacteria; *Escherichia coli* (ATCC 25922); *Pseudomonas aeruginosa* (ATCC 27853); *Enterobacter aerogenes* (ATCC 13048); *Acinetobacter hemolyticus* (ATCC 19002); *Klebsiella pneumoniae* (ATCC 13883); as well as two *Candida* species; *C. parapsilosis* (ATCC 22019), *C. albicans* (ATCC 10231) utilized in this study were kindly provided by Prof. Ali Osman Kiliç (Karadeniz Technical University, Trabzon, Turkey).

### Assessment of antimicrobial effects of the extract

The antimicrobial effects of the extract were assessed by agar well diffusion as described by Denev et al. [23]. Briefly, *M. smegmatis* was grown in BHIA; other bacteria were grown in MHA at 37°C for 72 and 24 h, respectively. *Candida* spp. was grown in PDA at 35°C for 48 h. Then, colonies of *M. smegmatis* were suspended in BHIB and the density was adjusted to 0.5 McFarland's standard [24]. The density of the other bacteria and *Candida* species were adjusted to 0.5 and 1.0 McFarland in a phosphate buffered saline solution (PBS), respectively.

Following this, *M. smegmatis* and other bacterial strains were spread onto BHIA and MHA plates, respectively, while the *Candida* species were seeded onto MHA plates that contained 2% glucose and 0.5 µg/ml methyl blue dye [25]. After the agar surface dried, the wells with 6 mm diameters were put on the plates. Then, 50 µl of the extract (stock solution 10 mg/ml in 3% DMSO) was carefully added to the respective wells. Ampicillin, gentamicin, ciprofloxacin and amphotericin-B were used as positive controls. As negative control, the equivalent amount of the DMSO was added to the corresponding wells. After incubation of the plates at 37°C or 35°C for each microorganism at appropriate times, the plates were observed for clear zone formation around the well. Those with a diameter greater than 6 mm were considered to be a zone of inhibition. All tests were repeated at least three times and the antimicrobial activity was expressed as the mean of inhibition zones (mm).

### Determination of minimum inhibition concentration (MIC) values

The MICs of the crude extract against the bacteria and fungi were determined using broth microdilution method. The microorganisms were incubated on the appropriate medium at appropriate times as indicated above. Subsequently, 0.5 McFarland suspensions for bacteria cultures and 1.0 McFarland from *Candida* cultures were prepared into appropriate medium: RPMI 1640/0.2% glucose for *Candida* species [26], BHIB for *M. smegmatis* [24] and MHB-II for other microorganisms were used [27,28]. For each microplate 100 µl respective medium was placed to each well, followed by the addition of 100 µl of extracts from the 10 mg/ml stocks to the first wells of the microplates. The positive controls (antibiotics) and the negative control (DMSO) were also added to the corresponding wells. Two fold dilutions were made starting from the first wells. Next, 10<sup>6</sup> CFU/ml bacteria to be tested were added to the respective wells and the microplates were incubated at 37°C for the relevant times for each microorganism. Each assay was performed in duplicate and repeated at least two times. On the basis of the colony counts, the MIC was defined as the lowest concentration of the extract providing complete inhibition of visible growth.

### Determination of minimum bactericidal concentration (MBC) values

To determine the minimum bactericidal concentration (MBC), 50 µl samples were taken out from the MIC well and the previous three wells and they were seeded onto the plates with relevant media and left to be incubated at 37°C for the relevant times for each microorganism. After evaluating the reproduction in petri dishes, MBC was defined as the lowest concentration at which no viable bacterial or fungal growth was present on the plate [29].

### STATISTICAL ANALYSIS

Results were presented as mean values and statistical analysis of the results was based on unpaired t-test analyses. The results with a p-value lower than 0.05 were considered statistically significant.

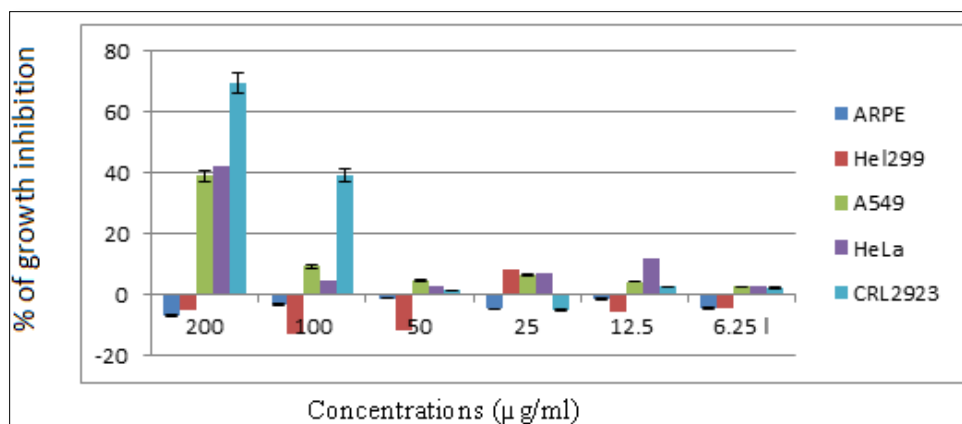
### RESULTS

#### Growth inhibition of human cell lines by the *C. sinensis* flowers extract

The antiproliferative effects of the different concentrations (200-6.25 µg/ml) of the EtOAc extract on human cell lines are presented in **Figure 1**. The results showed that at the highest concentration, the extract had no effect on non-transformed (ARPE-19, HEL-299) cells, while causing growth inhibition rates of 38.8%, 69.74% and 41.94% in the A549, CRL-2923 and HeLa cancer cell lines, respectively. The cytotoxic effect was dose-dependent. 200-100 µg/ml of extract exhibited the highest inhibition against the endometrial adenocarcinoma cells by causing 69.8% and

38.9% cell death, respectively. The differences between the normal cell lines and cancer cells were found to be significant at the highest concentration tested (200  $\mu\text{g/mL}$ )

( $p < 0.05$ ), with one exception in which significant inhibitory effect was observed for the endometrial adenocarcinoma cells at a concentration of 100  $\mu\text{g/mL}$  ( $p < 0.05$ ).



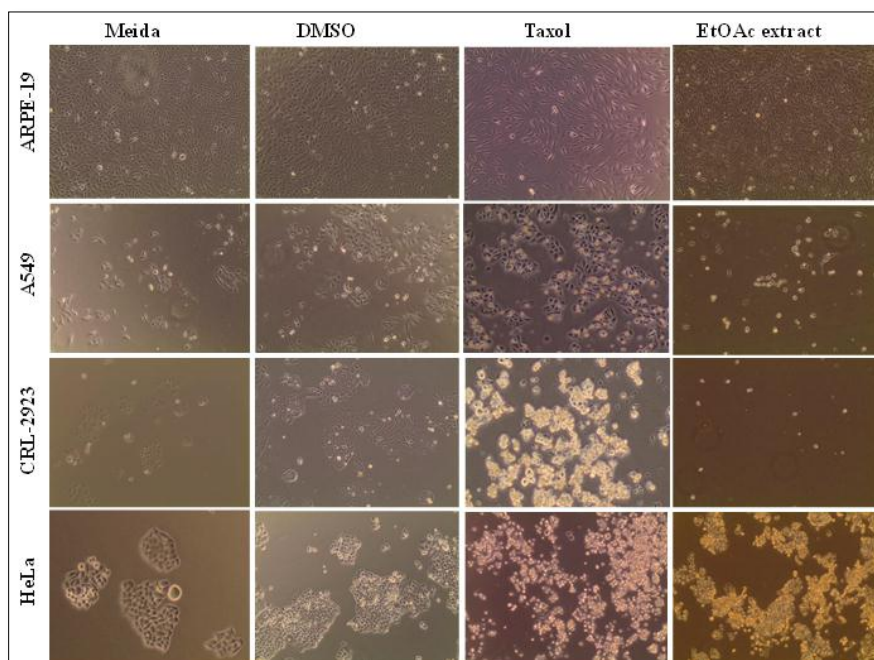
**Figure 1.** Antiproliferative effects of tea flower ethyl acetate extract.

Dose-dependent inhibitory effects of the *C. Sinensis* flowers extract on transformed (A549, HeLa, A549 and non-transformed (ARPE-19, HEL299) human cell lines. The cell was exposed to EtOAc extract or the equivalent amount of DMSO for 48 h. The results are presented as mean of at least three independent experiments with two replicates at each point

#### Findings on morphological changes

The morphological effects of the extract were determined after 24 h exposure to the extract (300  $\mu\text{g/mL}$ ), Taxol or

DMSO. As shown in **Figure 2**, no morphological changes were observed in the cell lines treated with the negative controls (DMSO or media). Meanwhile, the positive control Taxol (5 nM) caused rounding and detachment of the cancer cell lines (A549, HeLa, CRL2923), but there was no significant change in the morphology of the ARPE-19 cells. The extract (300  $\mu\text{g/mL}$ ) induced morphological alterations and showed a very high activity against all cancer lines without affecting the ARPE-19 cell line.



**Figure 2.** Morphological changes observed after incubation of the cell lines (ARPE-19, A549, CRL-2923 and HeLa) with medium, DMSO (negative controls), Taxol (positive control/5 nM) or extract (300  $\mu\text{g/mL}$ ) at 37°C for 24 h. Morphological changes of the cells were examined under an Olympus inverted microscope using 20x objective lens.

### Antimicrobial activity using the agar well diffusion method

The antibacterial activity of the extract is shown in **Table 1** and **Figure 3**. Regarding the inhibition zones, the extract had a lower zone inhibition diameter in for all microorganisms in comparison to the controls at 10 mg/ml. The diameters of the inhibition zones ranged from 10.66 mm to 17.33 mm for the microorganisms and 21.66 mm to 35.0 mm for the controls. *S. aureus* and *K. pneumoniae* were the most affected Gr(+) and Gr(-) bacteria, respectively (**Table 1**

and **Figure 3**). *B. subtilis* (11.66 mm) and *A. hemolyticus* (10.66 mm) were moderately susceptible, whereas the rest of the tested bacteria were unaffected by the extract. As indicated in **Table 1**, the extract exhibited inhibition zones of 19 mm and 21.33 mm diameters on *C. albicans* and *C. parapsilosis*, respectively, compared to the controls with a 28.66 mm inhibition zone diameter. In terms of antimicrobial activity, the extract may be more potent to *Candida* species compared to bacteria strains used in the study.

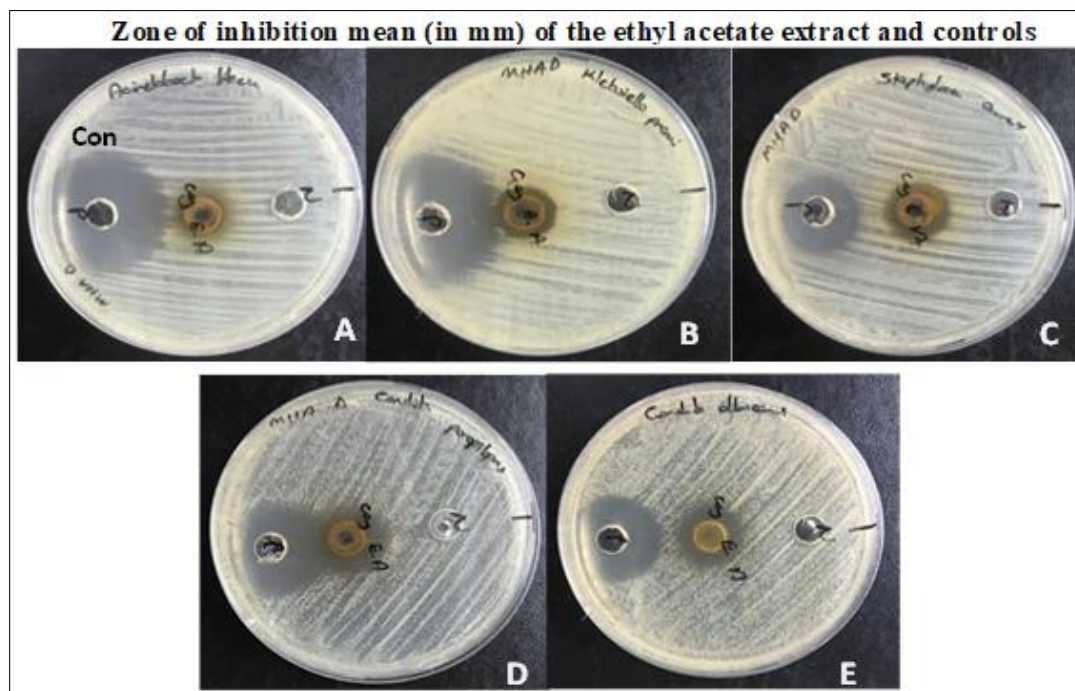
**Table 1.** Zone of inhibition mean (in mm) of the ethyl acetate extract and controls.

| Tested Microorganisms |                 |                 |      |      |      |                 |               |      |      |      |                 |                 |              |
|-----------------------|-----------------|-----------------|------|------|------|-----------------|---------------|------|------|------|-----------------|-----------------|--------------|
|                       | S.a             | B.s.            | E.f. | E.c. | P.a. | A.h.            | K.p.          | E.a. | S.t. | C.v. | C.a             | C.p.            | M.s.         |
| EtOAc.                | 17.33 ±<br>0.47 | 11.66 ±<br>0.47 | -    | -    | -    | 10.66 ±<br>1.24 | 15.0 ±<br>0.0 | -    | -    | -    | 19 ±<br>0.81    | 21.33 ±<br>0.94 | -            |
| Cons.                 | 21.66 ±<br>2.49 | 24 ±<br>1.41    |      |      |      | 35.0 ±<br>0.0   | 33 ±<br>1.41  |      |      |      | 28.66 ±<br>1.24 | 28.66 ±<br>0.47 | 27 ±<br>1.73 |

Values are expressed as Mean ± SD (n=3)

S.a.: *Staphylococcus aureus*; B.s.: *Bacillus subtilis*; E.f.: *Enterococcus faecalis*; E.c.: *Escherichia coli*; P.a.: *Pseudomonas aeruginosa*; A.h.: *Acinetobacter haemolyticus*; K.p.: *Klebsiella pneumoniae*; E.a.: *Enterobacter aerogenes*; S.t.: *Salmonella typhimurium*; C.v.: *Chromobacterium violaceum*; C.a.: *Candida albicans*; C.p.: *Candida parapsilosis*; M.s.: *Mycobacterium smegmatis*

(-) No inhibition; EtOAc: Ethyl acetate extract; Con: controls; DMSO (negative control), ampicillin, gentamicin, amphotericin B and ciprofloxacin (positive controls)



**Figure 3.** Zones with a diameter greater than 6 mm were considered zones of inhibition A: *A. hemolyticus*, B: *K. pneumoniae*, C: *S. aureus*, D: *C. albicans*, E: *C. parapsilosis*, P: positive control (ampicillin, gentamicin or amphotericin B), N: negative control, E.A: ethyl acetate extract.

### Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC)

Microorganisms with an inhibition zone diameter of  $\geq 15$  mm for the extract were used to test the MIC and MBC values. The means of three experiments were calculated and the results are presented in **Table 2**. The most sensitive bacterium was *S. aureus* with 62.25  $\mu\text{g/ml}$  and 125  $\mu\text{g/ml}$  MIC and MBC values, respectively. Additionally, all yeast

strains used in the study also exhibited MIC and MBC values of 62.25  $\mu\text{g/ml}$  and 125  $\mu\text{g/ml}$ , respectively. The MIC and MBC values for *K. pneumoniae* were found to be 500  $\mu\text{g/ml}$  and 1000  $\mu\text{g/ml}$ , respectively. These results agreed with the agar well diffusion results in which *S. aureus* and the yeast strains were more sensitive to the extract.

**Table 2.** Different microorganisms and respective MIC and MBC values.

| Microorganisms         | MIC* ( $\mu\text{g/ml}$ ) | MBC** ( $\mu\text{g/ml}$ ) |
|------------------------|---------------------------|----------------------------|
| <i>S. aureus</i>       | 62.25                     | 125                        |
| <i>C. albicans</i>     | 62.25                     | 125                        |
| <i>C. parapsilosis</i> | 62.25                     | 125                        |
| <i>K. pneumoniae</i>   | 500                       | 1000                       |

\*MIC: Minimum inhibitory concentration

\*\*MBC: Minimum bactericidal concentration

**Table 2** summarizes the MIC and MBC values for the ethyl acetate extract of *C. sinensis* flowers. The extract was effective against Gram positive *S. aureus*, Gram negative *K. pneumoniae* and displayed the MIC values of 62.25  $\mu\text{g/ml}$  and 500  $\mu\text{g/ml}$ , respectively. Moreover, its best activity was against the yeast with the MIC and MBC values of 62.25  $\mu\text{g/ml}$  and 125  $\mu\text{g/ml}$ , respectively.

### DISCUSSION

In the past decades, there has been a long-standing view that tea is good for health and can help reduce the risk of many cancers. In recent years, studies on cancer prevention of tea compounds have been extensively increased [30]. More recently, epidemiological studies have linked tea consumption to reduced risks of some cancers and marked the health promoting effects of tea [31-33]. Animal model studies revealed that consumption of green tea decreased the size of breast and prostate cancers [34]. Additionally, epidemiological studies since 1970s showed that, especially in areas where green tea is intensively consumed, breast, lung and gastritis cancers occur less frequently [12]. A review article presented in 2015 reported that using green tea catechins, EGCG (epigallocatechin gallate) in combination with current cancer drugs resulted in a synergistic effect of 70.3% reduction in tumor volume in animals [35].

In addition to their anti-carcinogenic properties, the antimicrobial effects of the tea components and extracts have been carried out by various studies. Their findings revealed a wide range of antimicrobial activity [36-38].

Most of the studies on the antiproliferative activities of tea plant have focused on the extracts or components obtained from the leaves. Even though it has been showed that tea flowers are rich in polyphenols, alkaloids, essential oils and polysaccharides [18,19] as far as we know, there are very

few studies showing the effects of the tea flowers extract or the components on human cancer cells and microorganisms. Tea leaf extract have been shown to inhibit growth of cells derived from cervical cancer (HeLa), hepatocellular carcinomas (HepG2), prostate cancer (DU145) and colorectal cancer (CaCo-2) [39-41]. Similarly, Yadav and Mendhulkar [42] also showed that after treatment of human colon (HT-29), breast MCF-7 and human leukemia (MOLT-4) cancer cell lines with *C. sinensis* tea leaves extracts caused growth inhibition. Other studies further reported that catechins obtained from tea leaves exhibited anticancer activity against human myeloid leukemia (TF-1a, MV4-11), human lung cancer (PC-9) and human prostate cancer (PC-3) cell lines [35,43,44].

The present study permitted the evaluation of antiproliferative and antimicrobial properties of the EtOAc extract obtained from *C. sinensis* flowers. Whole EtOAc extract obtained from the tea flowers exhibited strong antiproliferative activities against all cancer cell lines tested by inducing morphological alterations in those cells with no effect on non-transformed cells (**Figure 2**). Here, we demonstrated that treatment of human non-transformed (ARPE-19, HEL-299) and transformed (A549, CRL-2923, HeLa) cell lines with EtOAc extract obtained from the tea flowers resulted a dose-dependent cytotoxic effect on all cancer cells. In particular, at concentrations of 100-200  $\mu\text{g/ml}$  exhibited the highest growth inhibition against the endometrial adenocarcinoma (CRL-2923) cells, causing 69.8% and 38.9% cell death, respectively (**Figure 1**). As mentioned previously, very few investigations have been done with the tea flowers in terms of antiproliferative activity.

The study permitted the evaluation of antiproliferative and antimicrobial properties of the EtOAc extract obtained from

*C. sinensis* flowers. Whole EtOAc extract obtained from the tea flowers exhibited strong anti-proliferative activities against all cancer cell lines by inducing morphological alterations in those cells without affecting non-transformed cells (**Figure 2**). Here, we demonstrated that treatment of human non-transformed (ARPE-19, HEL-299) and transformed (A549, CRL-2923, HeLa) cell lines with EtOAc extract obtained from the tea flowers resulted in a dose-dependent cytotoxic effect on all cancer cells. In particular, at the concentrations of 100-200 µg/ml exhibited the highest growth inhibition against the endometrial adenocarcinoma (CRL-2923) cells causing 69.8% and 38.9% cell death, respectively (**Figure 1**). As mentioned previously, very few investigations have been done with the tea flowers in the term of antiproliferative activity. Way et al. [45] demonstrated that water extracts obtained from tea flowers caused a dose-dependent growth inhibition in human breast cancer cell line (MCF-7). Another study conducted in 2012 showed that polysaccharides from the flower of the tea plant exhibited anticancer activity against human gastric cancer BGC-823 [46].

Although there is a limited number of studies regarding the anti-proliferative activities of tea flowers in in vitro conditions, our results show similarity to those reported. Different extracts may have different anti-proliferative properties in different cancer cell lines. In this study, the tea flower extract was more potent and more selective against the endometrial cancer cell line (CRL-2923). This may be related to the composition of the extract. As far as we know, this study is the first report to investigate the antiproliferative activity of the EtOAc extract obtained from tea flowers. This extract may have to be further fractionated to assess the origin of selectivity.

Antibiotic resistance is a global problem because of multidrug-resistant pathogens. In recent years, there has been an increase in published reports showing antimicrobial activities of tea leaf extracts and compounds. In the present study, the antimicrobial effects of the EtOAc extract of the flowers were also evaluated. The extract had varying degree of antimicrobial effects on the microorganisms used. Among the Gr(+) bacteria, the extract showed the highest antimicrobial activity against *S. aureus* with the MIC and MBC values of 62.25 µg/ml and 125 µg/ml, respectively.

Reports published between 1997 and 2013 also indicated that extracts and some compounds obtained from the leaves of the tea plant induced antimicrobial activities against *S. aureus* [47,48]. Even though we used the flower extract of the tea plant we obtained similar results. This may indicate that the both flower and leaf of the tea may have some similar compound/compounds in their extracts. In the case of Gr(-) bacteria used in the study, *K. pneumoniae* was the most affected one with a 15 mm inhibition zone diameter and with an MIC value of 500 µg/ml. The effects of the extract on *K. pneumoniae* were important since there are

very few studies showing the growth inhibition of tea on *Klebsiella* spp. [36]. Two studies revealed that there was some variation in the effects of the extracts against different strains [49,50]. Similar to those above, our results also indicated that the EtOAc extract of tea causes growth inhibition in *K. pneumoniae*, which is an encapsulated bacteria, one of the most prominent opportunist bacteria in clinical infections and has recently gained resistance worldwide [51,52]. We believe this study is the first to show that the EtOAc extract of tea flowers induce specific antimicrobial activity against *K. pneumoniae* and that these have a good potential to be developed into useful treatments for *K. pneumoniae* infections.

The flower extract was also found to have low antibacterial effects on Gr(+) *B. subtilis* and Gr(-) *A. hemolyticus*. On the other hand, no antimicrobial activity of the extract was evident against *E. fecalis*, *E. coli*, *P. aeruginosa*, *E. aerogenes*, *S. typhimurium* or *C. violaceum*. Nevertheless, there is a body of reports indicating anti-bacterial effects of tea extracts especially against *E. coli* [36,48]. Among these, Wu et al. [47] used the water extract of tea leaves to investigate its antimicrobial activity. Their results revealed that the water extract did not induce any antimicrobial effect on Gr(-) *E. coli* similar to our results. On the other hand, a recent study reported that the water extract obtained from the tea leaves caused antimicrobial activity against Gr(+) bacteria (*S. aureus*, *Bacillus cereus*) and as well as Gr(-) *E. coli*.

One possible reason for the differences between our results and findings in the literature might be that, in this study, we used the extract of flowers, rather than leaves. A study that was published in 2015 reported that secondary metabolites may vary in different organs of *C. sinensis*. They showed that 3814 unigenes were expressed only in the flowers of tea in comparison to other tissues of the plant [6]. Further studies are needed to address the differences in antimicrobial effects against *E. coli*.

Fungal infections have been increasing due to drug resistance against currently available antifungal agents [53]. Therefore, new antifungal agents are needed. The antifungal activities of tea have been investigated by several researchers. Data from those studies showed that especially catechins obtained from the leaves of the tea plant exhibited significant activities against yeasts [53-55]. Similar to those, our results indicated that, the extract obtained from the flowers of tea caused strong antifungal activities. The mean zone of inhibition for *C. albicans*, *C. parapsilosis* and the control were 19 mm, 21 mm and 28.66 mm, respectively. The difference between the yeasts and the control were not found to be statistically significant. The MIC value of the extract for the *Candida* species was 125 µg/ml. In the literature, the ability to determine the MIC value of a plant extract in the range of 100 µg/ml-1000 µg/ml has been

reported as an indicator of the strength of the activity of that extract [56].

In conclusion, a large number of studies in the literature have investigated the antimicrobial and anticancer properties of the tea leaf. This study is the first report to show the efficacy of the EtOAc extract of tea flower against microorganisms and human cancer cells. Tea flower extracts may have antimicrobial and anticancer potentials, which depend on the types of microorganisms and cancer cell lines. Determination of the phyto-components in the extract obtained from the flowers of the plant and their possible antimicrobial and anticancer effects may lead the way to pharmacologically acceptable antimicrobial and anticancer agents

## CONCLUSION

The study shows that some substances in the extract may have an anti-carcinogenic and antimicrobial potential. Despite these encouraging results, we believe that extensive research is needed for determining the bioactive compounds in different extracts and their benefits for health. As a first report showing the anticancer and antimicrobial effects of the EtOAc extract from *C. sinensis* flower, the investigation may provide promising improvements in therapeutic approaches.

## ACKNOWLEDGMENT

This work was supported by Recep Tayyip Erdoğan University Research Fund Grants (BAP-TDK-2018-959). We would also like to give our special thanks to Prof. Dr. Bedia Ağaçhan Çakmakoglu for providing CRL-2923 cells, Prof. Dr. Fikrettin Şahin for providing HeLa and A549 cells, Assist. Prof. Dr. Muradiye Acar for providing the ARPE-19 cells.

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