Evaluation of biological activity of *Diplotaenia cachrydifolia* Boiss. that medicinal plant

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ABSTRACT

The aim of this study is to evaluate the antimicrobial, antioxidant, and antigenotoxic activity of extracts obtained from *Diplotaenia cachrydifolia* Boiss, which is traditionally used for food and medical treatment by the local people of Hakkâri Province (Turkey). As a result of this study, the extracts of *D. cachrydifolia* exhibited a broad antimicrobial spectrum and high antimicrobial activity. It was also observed that the extracts had a high antioxidant activity. The DPPH IC₅₀ values of the extracts were 2.5234 µg/mL, 6.0075 µg/mL, and 29.1974 µg/mL for ethanol, acetone, and hexane, respectively. The highest phenol content was found to be 55.36±0.035 µg/mL for ethanol. Furthermore, the ethanol extract was found to have a protective effect against mitomycin C-induced genotoxicity. *D. cachrydifolia* has a high biological activity; therefore, it is anticipated that future pharmaceutical studies will shed more light on this plant.

Keywords: Biological activity, genotoxicity, *Diplotaenia cachrydifolia*, DPPH, phenolic component

INTRODUCTION

In a modern context, at least 25% of the active ingredients of pharmacologically produced medicines are derived from plants. In addition, the active ingredients of many synthetically produced medicines are similar to chemicals that were first isolated from medicinal plants (Berber et al. 2013). The active ingredients in medicinal plants have been shown to exhibit high biological capability (Bhouri et al. 2010; Kilani-Jaziri et al. 2011) and these plants seem to be the best resources for the discovery of novel bioactive chemicals in the design of new drugs. Recently, there has been considerable interest in the antimicrobial (Rates 2001), antioxidative (Kilani-Jaziri et al. 2011), and antigenotoxic activities (Amkiss et al. 2013) of medicinal plants.

At present, alternative antimicrobial medicines are needed, in particular, for the treatment of infectious diseases. Mounting resistance of microorganisms to many antibiotics has led to increasing difficulties in the treatment of infectious diseases. In this context, local medicinal plants are known to be rich in antimicrobial properties and thus, a source of novel antimicrobials (Rates 2001; Kilani-Jaziri et al. 2011). Furthermore, some medicinal plants are reported to exhibit high antioxidant activity (Kilani-Jaziri et al. 2011), and plant phenolics are generally the source of these natural antioxidant properties (Atoui et al. 2005; Cavlak and Yagmur 2016). Antioxidants prevent the formation of free radicals produced routinely in all cells as part of their normal function; however, free radical accumulation in cells may play a role in many diseases (Young and Woodside 2001). Recently, there has been a growing interest in natural antioxidants of plant origin. Natural antioxidants obtained from medicinal plants can prevent the formation of free radicals, thus indicating their potential as novel therapeutic agents (Nogochi and Nikki 2000; Chaabane et al. 2012). Of late, considerable research has been focused on the antigenotoxic activity of medicinal...
plants (Ankiss et al. 2013). With the biosphere progressively accumulating chemical compounds that possess mutagenic properties (Karamova et al. 2011) and the increase in health problems associated with human exposure to environmental mutagens, the importance of antigenotoxicity has been highlighted (Demir et al. 2013). Moreover, recent studies seem to indicate that medicinal plants hold promise for the development of antigenotoxic agents.

Medicinal plants seem to be the best resources for the discovery of new drugs and so novel medicinal plants are needed for the control of different diseases. In this context, the main aim of this work was to reveal the biological importance of *D. cachrydifolia* Boiss. which is known as a medicinal plant. This plant, known locally as “siyabu”, flourishes at high altitudes and grows wild in Hakkâri Province (Turkey). *D. cachrydifolia* has been used by the local inhabitants for medicine as treating diabetes, rheumatism and scorpion stings and food for many years and is considered to be one of the most important plant species (Kaval et al. 2014; Uce and Tuncturk 2014). Kaval et al. (2014) were the first to report the medicinal uses of *D. cachrydifolia* Boiss. The root and aerial parts of the plant are used for medical applications among local people (Agaoglu et al. 2005; Uce and Tuncturk 2014) and according to them, the plant has properties as an anti-snake venom properties. The parts growing above the ground are used for food, while the roots are used for medicine. Locals have reported that, in addition to snakebites, *D. cachrydifolia* is used traditionally for treating diabetes, rheumatism and scorpion stings (Agaoglu et al. 2005; Mükemre et al. 2015). Consequently, in this study, the use of this plant was evaluated from different pharmacological perspectives. There is also the possibility that this plant will gain value for use in different pharmacological areas.

**MATERIALS AND METHODS**

**Plants Used in the Study**

In previous studies, the potential medical properties of *Diplotaenia cachrydifolia* Boiss. were identified through its use among the population (Kaval et al. 2014; Uce and Tuncturk 2014). In April-May 2016, it was purchased from the local public markets in Yüksekova, Hakkâri. The plant materials were identified by S. Semra Candar Erol, in the Traditional and Complementary Medicine Research Center, Düzce University. The freshly obtained *D. cachrydifolia* samples were minced and dried in a drying oven with the temperature kept under 40°C and then pulverized in a grinder. The powdered plants were then mixed at a 1:10 ratio with hexane, ethanol and acetone solvents, consecutively and extruded at 150-170 rpm for 24 h at room temperature. After each extraction, filtration was carried out and the remaining plant residue was dried for use in the next solvent. The obtained extracts were evaporated under vacuum at 80-150 rpm at about 30°C. The quantities of the extracts obtained after evaporation were determined and dissolved in dimethyl sulfoxide (DMSO, Merck). DMSO is commonly used to prepare stock solutions of samples in a solvent in which is highly soluble because it is a polar and aprotic solvent. These samples were stored at +4°C for further use in subsequent stages of this research (Baravalia et al. 2009).

**Percentage of extract yields**

The yield of the extracts was calculated using the following % yield formula:

\[
\text{% Yield} = \left( \frac{w_f - w_i}{w_i} \right) \times 100
\]

where \(w_i\) is the initial weight of the dried plant sample, \(w_f\) = the weight of the cabinet and \(w_r\) = the weight of the remaining extract after evaporation (Anokwuru et al. 2011).

**Microorganisms used in the study**

The microorganisms used in the antimicrobial activity test studies were provided by Düzce University Faculty of Agriculture and Natural Sciences, Field Crops Department Microbial Biotechnology Laboratory and the Faculty of Medicine Microbiology Laboratory, and included: *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Versinia pseudotuberculosis* ATCC 911, *Proteus vulgaris* ATCC 13315, *Salmonella typhimurium* ATCC 14028, *Enterococcus faecalis* ATCC 29212, *Enterobacter cloacae* ATCC 13047, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 35218, *Listeria monocytogenes* ATCC 7644 and *Candida albicans* ATCC 90028. The microorganisms used in the study were grown at 37°C for 16-18 h using Mueller Hinton broth (MHB) or Mueller Hinton agar (MHA) media as described for use in antimicrobial activity tests (Sen and Batra 2012).

**Antimicrobial activity test**

The antimicrobial activity of the plant extracts was determined using the agar well diffusion method (Chung et al. 1990). Initially, test microorganisms were inoculated into the MHB medium and incubated for 16-18 h at 37°C in a shaking water bath. At the end of the incubation period, the absorbance of the microorganism cultures was measured in a spectrophotometer (Mapada, UV3100PC) at a wavelength of 600 nm. The cultures were then diluted with sterile dH₂O to about 1×10⁷-1×10⁸ CFU/mL. The 100 µL diluted microorganism samples were spread onto plate containing the MHA growth medium and then wells were opened on these plates. And finally, 100 mL of the hexane, ethanol and acetone extracts were added to each well. An antibiotic (Streptomycin, 10 µg/disc) was used as a positive control and dimethyl sulphoxide (DMSO) was used as a negative control (Arullappan et al. 2009). DMSO, which is not toxic for microorganisms, was used for preparation of test samples (Sokmen et al. 2013).

**DPPH radical scavenging activity**

The radical scavenging activity assay was performed using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical (Brand et al. 1995; Ertürk et al. 2014). For the study, the methanol solution of the DPPH radical was freshly prepared, and 1500 µL of the DPPH solution was added to 750 µL of plant extracts prepared at concentrations of 20-100 µg/mL. Absorbance values were measured after 30 minutes at 517 nm in the spectrophotometer. Butylated hydroxyanisole (BHA, Aldrich) was used as the positive control (standard). As the negative control, a mixture of 1500 µL of DPPH and 750 µL of methanol was used, while methanol was used as the blank. The process was repeated in duplicate. The DPPH radical scavenging activity was calculated using the following formula:
DPPH Radical Scavenging Activity (%) = \((A_0 - A_1) / A_0\) \times 100

\(A_0\) = the negative control absorbance value and \(A_1\) = the standard and sample absorbance value.

Total phenolic component analysis

The Folin-Ciocalteu reagent was used to determine the total phenolic component content of the extracts. The amounts of phenolic compounds corresponding to the gallic acid standard of the extracts were determined in the study (Döger 2010). The work was performed in triplicate at two different times.

**Allium cepa assay and treatments**

Healthy *A. cepa* L. (2 n=16), approximately equal in size, were used as the test material in the study. The outer dry skin and the old roots were carefully removed from the onions, then washed, dried and kept refrigerated at 4°C until the study began. Five onions were used for each concentration, including a negative and positive concentration. Three types of treatment groups were created: pre-, post- and simultaneous. In the pre-treatment group, the roots were first treated with *D. cachrydifolia* ethanol extracts at different concentrations (10, 100, 1000 μg/mL) and after 24 h were treated with Mitomycin C (MMC) at 5x10^-5 M concentration. In the post-treatment group, the roots were first treated with MMC at a concentration of 5x10^-5 M for 24 h, and then with the plant extracts at different concentrations (10, 100, 1000 μg/mL) for 24 h. In the simultaneous treatment group, MMC at a concentration of 5x10^-5 M was added to each concentration of the extract. In this study, MMC at a concentration of 5x10^-5 M was used as the positive control and distilled water as the negative control.

**Allium cepa chromosomal aberration assay**

With all treatments, root tips were fixed in Carnoy’s solution (3 acetic acid:1 methanol) for 24 h, placed into 70% alcohol and stored at 4°C. To prepare the formulations, the root tips were passed through distilled water, and then hydrolyzed with 1 N HCl for 15 min at 60°C. The root tips were stained with aceto-orcein for 20 min and crushed samples were prepared (Darlington and La Cour 1979). At least 1000 cells per well were examined for each concentration and for the controls. To determine the mitotic index, 3000 cells were counted, and the ratio of mitotic cells per 100 was calculated.

The percent of inhibition was calculated according to the formula:

\[ PI = \frac{a-b}{a-c} \times 100 \]

where \(a\) is the number of aberrant cells caused by the positive control, \(b\) is the number of aberrant cells caused by the ethanol extract of *D. cachrydifolia*, and \(c\) is the number of aberrant cells caused by the negative control (Kumari et al. 2013).

The linear relationship between the effects and the doses of the *D. cachrydifolia* via Pearson regression and correlation analyses is shown in Figure 1.

**Statistical Analysis**

The data obtained as a result of genotoxicity and cytotoxicity studies were analyzed by using The Statistical Package for the Social Sciences (SPSS) 20 for Windows (IBM Corp.; Armonk, NY, USA) and results obtained were expressed as mean±standard error (SE). The Kruskal-Wallis test was carried out followed by the Mann-Whitney U test to compare the statistical significance of the differences between the treated and control groups. The dose response relationship was determined using Pearson correlation analysis. A value of \(p<0.05\) was considered significant. The value for all statistics was \(p<0.05\) and for Mann Whitney U test, Bonferroni correction was selected as alpha/number of comparisons = 0.05/10 = 0.005. The dose response relationship was determined using Pearson correlation analysis.

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**Figure 1.** Percentage of inhibition of the genotoxic effect induced by MMC in the *A. cepa* chromosomal aberration method and the relationship between different concentrations of *D. cachrydifolia* ethanol extracts.
RESULTS

The percentage yields of the extracts obtained as a result of the study, which are given in Table 1, show the highest yield was achieved with the ethanol extract. Total phenolic component contents of the plant extracts are shown in Table 1 as the gallic acid equivalent (GAE, μg/mL). The total phenolic component content was found to be highest in the ethanol extract of the plant.

The zones of antimicrobial activity of the extracts against the test microorganisms are shown in Table 2 and Figure 2. The plant ethanol extract showed high antimicrobial activity against all test microorganisms used in the study. The acetone extract showed a high activity against all microorganisms except for P. vulgaris. The antimicrobial activity against P. vulgaris was relatively low. When the plant extracts were evaluated, high antimicrobial activity was observed against K. pneumoniae, L. monocytogenes, S. typhimurium, Y. pseudotuberculosis, P. aeruginosa, E. cloacae, S. epidermidis and C. albicans.

The DPPH radical scavenging activity of the extracts is shown as inhibition % in Figure 3. The sequence of the control and

| Table 1. Percentage yield and total phenolics content of extracts |
|------------------|-----------------|-----------------|
| Extracts        | Yield (%)       | Total Phenolic Content |
| Hexane          | 2.1205          | 43.59±0.657     |
| Ethanol         | 3.5972          | 55.36±0.035     |
| Acetone         | 0.3253          | 16.29±0.006     |

* µg gallic acid equivalent

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<th>Table 2. Antimicrobial activity test</th>
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<td>Microorganisms</td>
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<td>S. typhimurium</td>
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* µg gallic acid equivalent

Figure 2. a-d. The inhibition activity of extracts from D. cachrydifolia against the microorganisms (a), and different samples (b, c and d).
the extract activity was found as: BHA > ethanol extract > acetone extract > hexane extract. According to the inhibition % data, the DPPH radical scavenging activity closest to the standard BHA was observed in the ethanol extract of the plant. The \( \text{IC}_{50} \) values were calculated with linear regression analysis as 29.1974 μg/mL for hexane extraction, 2.5234 μg/mL for ethanol extraction and 6.0075 μg/mL for acetone extraction. When the results of the antioxidant activity analysis were evaluated, the radical scavenging activities of the ethanol and acetone plant extracts were high. Specifically, when the results of DPPH analyses were evaluated, it was seen that the second place in the order, in which ethanol extract had the highest antioxidant activity, was taken by the acetone extract.

The data obtained as a result of genotoxicity and cytotoxicity studies are shown in Table 3. Using the A. cepa chromosomal aberration method, the antigenotoxic effect of different concentrations of D. cachrydifolia against genotoxicity induced by MMC was evaluated. The MMC caused a variety of chromosome aberrations such as irregular metaphase, stickiness, c-mitosis, pole deviation, micronucleus, lagging chromosome and bridge (Figure 4). When all treatment models of D. cachrydifolia extracts were compared with the positive control group, there were reductions at the chromosomal aberration frequency. According to statistical analyses, statistical differences in the percentage of the mitotic index were found in the 100 μg/mL and 1000 μg/mL pre-treatment, and in the post-treatment highest concentration of 1000 μg/mL, while in the simultaneous treatment, there were a number of aberrations at all concentrations. Among all treatment models, it was determined that the maximum reduction in chromosomal aberration frequency occurred with the highest concentration (1000 μg/mL) of the post-treatment. Pearson correlation analysis showed that the percentage of inhibition of chromosomal aberration was linked to the concentration (Figure 3).

**DISCUSSION**

The availability of more than half a million identified plants in the world is promising because the medicinal activities of most plants have not yet been studied. Treatments stemming from current and future research will be determined by the properties of these medicinal plants (Rasool Hassan 2012).

As a result of this study, D. cachrydifolia was seen as a good antimicrobial source. Furthermore, the radical scavenging activities of the ethanol extracts were high. The Folin–Ciocalteu assay, based on the chemical structure of the phenolics (Atoui et al. 2005), found the total phenolic component content to be highest in the ethanol extract of the plant. Thus, it can be said that the radical scavenging activity of these plant extracts can indicate their total phenolic content. Furthermore, this is consistent with the results of the antioxidant activity and antimicrobial activity of the plant extracts.

Results showed the ethanol extract of D. cachrydifolia to have the highest biological activity. There are very few studies in the literature on the chemical characteristics of D. cachrydifolia, and no studies on its biological activity or antimicrobial and antioxidant properties. A previous study investigated the essential oil yield
and composition of the aerial parts of *D. cachrydifolia* and reported that the high amount of the oil in its green state is a factor for decreasing plant palatability (Azarnivand et al. 2009). Harkiss and Salehy Surmaghy determined the chemical composition of *D. cachrydifolia* (Harkiss and Salehy Surmaghy 1988), while the antimicrobial activity of the essential oils obtained from the leaves, root and the seeds of *D. damavandica* was determined in another study. That study reported that the essential oil from the leaves displayed the highest antimicrobial activity against all test microorganisms including *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* (Eftekhari et al. 2005). This study also reported that *D. cachrydifolia* exhibited high antimicrobial activity against all test microorganisms including *K. pneumoniae*, *L. monocytogenes*, *S. typhimurium*, *Y. pseudotuberculosis*, *P. aeruginosa*, *E. cloacae*, *S. epidermidis* and *C. albicans*. These findings raise the possibility that members of this genus may be good sources of antimicrobial agents.

In yet another previous study, the DPPH radical scavenging activity of some species of the Apiaceae family was tested (Coruh et al. 2007). Since *D. cachrydifolia* is a member of the same family (Khajeh et al. 2012), the results are important for the present study. It was reported in another earlier work that the antioxidant capacities of *P. ferulacea*, *H. persicum* and *C. macropodum* had been determined for DPPH radical scavenging as IC$_{50}$ values of 0.242 mg/ml, 0.438 mg/ml and 0.623 mg/ml, respectively (Coruh et al. 2007). In the present study, the antioxidant capacities of *D. cachrydifolia* were calculated as IC$_{50}$ values of 29.1974 µg/mg, 2.5234 µg/mg and 6.0075 µg/mg for the hexane, ethanol and acetone extractions, respectively. The antioxidant activity of *D. cachrydifolia* was much higher compared with the results of the previous study. Furthermore, in the previous study, the total phenolic content of *P. ferulacea*, *C. macropodum*, and *H. persicum* was reported as 65.1±6.4, 34.0±7.0 and 59.6±2.8 µg/mg, respectively (Coruh et al. 2007). In the present study, the total phenolic content of *D. cachrydifolia* was calculated as 43.59±0.657, 55.36±0.035 and 16.29±0.006 µg/mg for the hexane, ethanol and acetone extractions, respectively, and it was observed that these results were very close to each other. It can be said that the antioxidant activity of *D. cachrydifolia* was higher because its active molecules were different from those of the phenolic substances.

The antigenotoxic activity of *D. cachrydifolia* was determined with the *A. cepa* chromosomal aberration assay according to Lima et al. (2017), compounds that prevent mutagenic or genotoxic events from affecting DNA can act by various mechanisms which are usually detected in pre-treatment or co-treatment. Other substances are effective by promoting DNA repair after damage has occurred and can be detected in post-treatment. No study is found in the literature that deals with the antigenotoxic potential of *D. cachrydifolia*. To detect the activity mechanisms of *D. cachrydifolia*, three different treatment groups were used in the present study. A protective effect was observed at these concentrations. The establishment of a protective effect indicated the presence of antimutagenic components in the *D. cachrydifolia* ethanol extract that acted as desmutagens. In other words, antimutagenic substances acted directly on the compounds that cause DNA mutations. This can also be accomplished by chemical or enzymatic means by inhibiting the metabolic activation of the pro-mutagenic or reactive molecules (Kada et al. 1978). Many antimutagenic components found in foods have antioxidant properties and work with pre- or simultaneous treatment to differentiate the free oxygen radicals that cause DNA mutations (Silva et al. 2013).
It should be mentioned in particular that plants from the family Apiaceae are commonly used as food and for flavoring as well as for medical purposes (Christova-Bagdassarian et al. 2013; Christova-Bagdassarian et al. 2014) and that scientific studies have supported the medical use of *D. cachrydifolia*.

**CONCLUSION**

This study showed that the extracts of *D. cachrydifolia*, specifically an ethanol extract, have a high antimicrobial and antioxidant activity. Therefore, it can be used as a natural source in different areas of the medicine and food industries. Furthermore, this work showed that the ethanol extract of *D. cachrydifolia* may act as desmutagens due to antimutagenic components. The results of this study have revealed the potential of the scientifically proven biological properties of *D. cachrydifolia*, which is routinely used for food and medical treatment by the local population of Turkey. In this respect, it can light the way for future pharmacological research on this plant.

**Peer-review:** Externally peer-reviewed.


**Conflict of Interest:** The authors have no conflict of interest to declare.

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