ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:3 (2017) 270-284

records of natural products

Antioxidant, Antigenotoxic, Antimicrobial Activities and Phytochemical Analysis of *Dianthus carmelitarum*

Rezzan Aliyazicioglu[1], Selim Demir[2*], Merve Badem[3], Sila Ozlem Sener[3], Nuriye Korkmaz[1], Elif Ayazoglu Demir[4], Ufuk Ozgen[3], Sengul Alpay Karaoglu[5] and Yuksel Aliyazicioglu[6]

[1]*Department of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080, Trabzon, Türkiye*
[2]*Department of Nutrition and Dietetics, Faculty of Health Science, Karadeniz Technical University, 61080, Trabzon, Türkiye*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, 61080, Trabzon, Türkiye*
[4]*Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080, Trabzon, Türkiye*
[5]*Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, 53100, Rize, Türkiye*
[6]*Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Türkiye*

(Received June 8, 2016; Revised November 9, 2016; Accepted November 13, 2016)

Abstract: In this study, we investigated the phytochemical composition, antioxidant, antimicrobial, and antigenotoxic properties of the aqueous extract of *Dianthus carmelitarum* for the first time. The phenolic and volatile compounds, antioxidant, antimicrobial and antigenotoxic activities of the extract were determined by HPLC and SPME-GC-FID/MS, spectrophotometric, agar well diffusion methods and comet assay, respectively. The polyphenolic content and ferric reducing power values of the extract were found 12.6 ± 0.27 mg gallic acid and 238 ± 2.89 µM trolox equivalents per g sample, respectively. Syringaldehyde and chlorogenic acid were detected as major phenolic compounds, while terpenes were determined as major volatile compound. *Dianthus carmelitarum* extract especially exhibited moderate antimicrobial activity against *Mycobacterium smegmatis*. Extract reduced H_2O_2-induced DNA damage in a concentration dependent manner in fibroblast cells compared to positive control (only 20 µM H_2O_2 treatment). *Dianthus carmelitarum* can be considered in the food, cosmetic, and drug industries due to its antioxidant, antimicrobial, and antigenotoxic activities.

Key words: Antimicrobial activity; antioxidant effect; chromatography; *Dianthus carmelitarum*; genotoxic stress.

1. Introduction

Dianthus which is a medicinal plant, belongs to family of *Caryophyllaceae* and includes more than 300 species [1]. There have been reported 76 *Dianthus* species from Turkey, and 33 species of them are endemic (including *Dianthus carmelitarum*) [2,3]. *Dianthus* species have been used in traditional medicine to treat chronic pains, urinary infections, carbuncles, menostasis, gonorrhea, cough, liver diseases, and some types of cancer [1,4]. *Dianthus* species are rich in phenolic (kaempferide, quercetin, kaempferol, apigenin, luteolin, acacetin, naringenin, and their glycosides), and volatile compounds (monoterpene and sesquiterpene hydrocarbons) [1,5]. *Dianthus* species and their isolated compounds also exhibit antibacterial, antifungal, cytotoxic, antioxidant, and antidiabetic activities due to the above mentioned compounds [4,6-8].

Oxidative stress, which is the corruption case of the balance between reactive oxygen species (ROS) formation and antioxidant defense in the direction of the oxidants and leads to cellular damage.

* Corresponding author: E-Mail: selim-demir@hotmail.com; Phone:+90 462 377 88 41, Fax: +90 462 325 28 21

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 04/29/2017 EISSN:1307-6167

DNA is an important target of ROS, and ROS induced-DNA damage is in a close relationship with many pathological situations, such as cancer, cardiovascular diseases, and diabetes. Plants involve many antioxidant components, such as polyphenolic and volatile compounds, and these compounds preserve cells against the deleterious effects of ROS. The antioxidant effect of these compounds is explained to their ability to donate electrons to ROS, chelating metal ions, and stimulate antioxidant enzymes. In recent years, exploration of new natural antioxidants is therefore a popular research area in world, and traditional medicine is seen as a starting point for new discoveries [9,10].

Pathogenic bacteria are considered as a main reason of morbidity and mortality in people. Although many antibiotics are produced by drug industries in every year, resistance to these drugs by microorganisms has increased day by day. Due to the increase of resistance to antibiotics, new strategies are needed to overcome this problem. Plants are considered as substantial source of new antimicrobial agents due to both their useful properties, such as low toxicity, safety, efficiency, and using in the treatment of infectious diseases in folk medicine for centuries [11,12].

Phytochemical characterisation and various biological activities of different *Dianthus* species have been demonstrated by some reports before. For instance, Ding *et al.* isolated some compounds, such as benzoic acid, kaempferol, quercetrin, 3,5,7-trihydroxy-3',5'-dimethoxyflavone, trans-*p*-coumaric acid from *D. superbus* [13], while Yu *et al.* demonstrated that antioxidant and cytotoxic properties of various fractions of ethanolic extract of *D. superbus*. Especially, ethyl acetate fraction shows strongest antioxidant activity than the others according to DPPH radical, superoxide anion, and hydroxyl radical scavenging activity, total phenolics, and reducing power assays. Moreover, ethyl acetate fraction exhibits antiproliferative activity in human liver cancer cells through inducing mitochondrial apoptosis pathway and caspase activity in the same study [4]. In addition, ethanolic extract of *D. coryophyllum* shows moderate antibacterial effect against *E. coli*, *B. subtilis*, *S. aureus*, *S. epidermidis*, and *P. aeruginosa* and antifungal effect against *A. niger* and *C. albicans* [6]. Interestingly, it is reported that hydroethanol extract of *D. basuticus* can cause alterations in food and water consumption, but produce no consistent change in haematological, biochemical, and histopathological parameters in rats [14]. The result of mentioned study may be seen a starting point for various *in vivo* trials of *Dianthus* extracts. Hovewer, there is no study about neither phytochemical composition nor biological effect of *D. carmelitarum* in the literature. Therefore, the purpose of the current study was to determine phenolic and volatile composition, antioxidant, antimicrobial, and antigenotoxic activities of aqueous extract of *D. carmelitarum* for the first time.

2. Materials and Methods

2.1. Reagent

Folin reagent, methanol, ethanol, sodium carbonate, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride, potassium acetate, trolox, 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), agarose, sodium chloride, sodium hydroxide, hydrogen peroxide (H_2O_2), ethylenediaminetetraacetic acid, tris base, triton X-100, trypan blue, butylated hydroxytoluene (BHT), and phosphate buffer saline (PBS) tablets were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, acetic acid, hydrogen chloride, and dimethyl sulfoxide (DMSO) from Merck (Darmstadt, Germany). All solvents used were HPLC grade. Chemicals and reagents were ACS grade or higher. Mueller Hinton (MH) broth, brain heart infusion agar (BHA), and sabouraud dextrose agar (SDA) was from BD Difco (Detroit, MI, USA). Penicillin-streptomycin and trypsin from Biological Industries (Kibbutz Beit HaEmek, Israel). Eagle's minimum essential medium (EMEM) and fetal bovine serum (FBS) was purchased from Lonza (Verviers, Belgium) and Biochrom (Berlin, Germany), respectively.

2.2. Plant Material and Extraction

Samples of *D. carmelitarum* were collected from Sinop, Turkiye. Voucher specimen was deposited at the herbarium of the Faculty of Pharmacy, Ankara University, Turkiye with the herbarium number of AEF-26695. The aerial parts of the plant were air-dried at 25ºC for 20 days and powdered using blender

and milling into fine powder. The powder of the plant material was stored as packed in freezer bags at -20°C until tested. For preparing stock aqueous extract of *D. carmelitarum*, 1 g powder of the plant material was weighed and mixed with 20 mL distilled water and then mixture was continuously stirred at 25ºC for 24 h. After that, the suspension was removed by centrifuging at 10000×g for 15 min. Then, the supernatant was concentrated using a rotary evaporator (IKA-Werke RV05 Basic, Staufen, Germany). A far amount residue was diluted with methanol, was filtered with 0.45 µm filter, and was used for HPLC analysis of phenolic compounds. The remained dry residue was resolved with distilled water and filtered with 0.2 µm filter then stored in 4ºC until used for antioxidant, antimicrobial, and antigenotoxicity experiments.

2.2.1. Estimation of Total Phenolic Content (TPC)

Content of total phenolics of the extract was established by the spectrophotometric method [15] using gallic acid as a standard. The quantity of phenolic compounds was indicated as mg of gallic acid equivalents (GAE)/g sample.

2.2.2. Ferric Ion Reducing Antioxidant Power (FRAP)

The reducing power of the extract was established by FRAP assay [16] using trolox as a standard and the results were indicated as µM trolox equivalents (TE)/g sample.

2.2.3. Free Radical Scavenging Activity

The free radical scavenging activity of the extract was established by DPPH assay [17] using BHT as a standard antioxidant compound. The percent reduction of the DPPH radical was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = 100 - (A_{sample}/A_{control}) \times 100$$

The SC_{50} value (the concentration of compound required to reduce the absorbance of DPPH by 50%) was estimated graphically in five different concentrations. SC_{50} value of extract was stated as mg/mL.

2.2.4. Cupric Reducing Antioxidant Capacity (CUPRAC*) Assay*

Cupric reducing antioxidant capacity of extract was determined using their cupric ion reducing capability in the presence of neocuproine [18] and results were indicated as µM trolox equivalents (TE)/g sample.

2.3. Determination of Phenolic Compounds by RP-HPLC

Thirteen standards were used for HPLC analysis as follows; gallic acid, protocatechuic acid, protocatechuic aldehyde, *p*-OH benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, syring aldehyde, *p*-coumaric acid, ferulic acid, sinapic acid, and benzoic acid. Previously, a stock solution of each standard (50 µg/mL) was prepared and filtered through 0.45 µm membranes. A stock solution was prepared by mixing all standard solutions (50 µg/mL). To make calibration curve, the stock solutions of mixed standards were diluted in the concentrations range of 2.5-50 µg/mL.

HPLC analysis of phenolic compounds was performed using a reverse phase column (250 × 4.6 mm i.d, 5 µm) (Waters Spherisorb, Milfort, MA, USA), on a gradient program with two solvents system [A: 2% acetic acid in water; B: 0.5% acetic acid in acetonitrile:water (1:1)] at a constant solvent flow rate of 1.2 mL/min on a HPLC system (Shimadzu Corporation, LC 20 AT, Kyoto, Japan). Injection volume was 20 µL. The signals were detected at 232, 246, 260, 270, 280, 290, 308, and 328nm by diode array detector (DAD) detection with column temperature at 25ºC. The determination of phenolic compounds of the extract was carried out using the same HPLC conditions. Peaks were detected by comparing retention time of known phenolic standards. To verify the repeatability of the method, the extract was run triplicates. The method validation was evaluated in terms of linearity, detection limits, quantification limits, recovery, precision, and selectivity according to the ICH guidelines [19]. At least five different

concentrations of phenolic compounds mixture solution in the range of 2.5-50 µg/mL were analyzed in five replicates. The peak areas were plotted against each concentration of the mixture solutions to establish a linear regression equation and to identify value of correlation coefficient. The values of limit of detection (LOD) and limit of quantification (LOQ) were assigned using the signal-to-noise method. A signal-to-noise ratio (S/N) of threefold is accepted to evaluate LOD and signal-to-noise ratio of tenfold is used for to evaluate LOQ. Three different concentrations of mix solutions (5, 25, and 50 µg/mL) were applied triplicates for determination of recovery. The recovery was evaluated as mean and standard deviation of percent known amount. The intra-day and inter-day precision were identified as the relative standard deviation (%RSD) of retention times and % peak areas with three different concentrations, three replicates by the standards mix solutions for two distinct day. The method selectivity was appraised by the resolution study betwen the standarts peaks.

2.4. Solid-Phase Microextraction (SPME)

A manual SPME apparatus including fiber (polydimethylsiloxane/divinylbenzene) was obtained from Supelco (Pennsylvania, USA). The SPME fibers were conditioned for 5 min at 250°C inside the GC injector. Subsequent assays were performed on the basis of a conditioning time of 4 min of desorption following all extractions. Extractions were carried out at 50°C using incubation and extraction times 5 and 10 min, respectively. Each sample was analyzed individually and means were calculated.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS)

GC analysis performed using a gas chromatography device (Shimadzu GC 2010 Plus, Kyoto, Japan) attached to a mass selective detector (Shimadzu QP2010 Ultra, Kyoto, Japan) according to previously described method [20]. Separation was carried out using a Restek Rxi-5MS capillary column (Bellefonte, PA, USA) 60 m length, 0.25 mm i.d. and a 0.25 µm phase thickness in split mode. Carrier gas was helium (99.99%) at a constant flow rate of 1 mL/min. Volatile compounds were compared to their retention index (RIs) (relative to C7-C30 alkane standards) for identification. Mass spectral data were compared to those held in the FFNSC1.2 and W9N11 library of mass spectra. Finally, all findings were compared against the relevant data in the literature [21].

2.6. Antimicrobial Activity Assessment

All tested microorganisms were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey). These were *Escherichia coli* (ATCC 25922), *Yersinia pseudotuberculosis* (ATCC 911), *Pseudomonas aeruginosa* (ATCC 43288), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus 709 ROMA, Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 60193), and *Saccharomyces cerevisiae* (RSKK 251).

Simple susceptibility screening test using agar-well diffusion method as adapted earlier was performed [22]. All bacteria were suspended in MH broth except *M. smegmatis* which was augment in BHA. For yeast-like fungi SDA was used. Each microorganism was diluted to approximately 10^6 colony forming units per mL. They were "flood-inoculated" onto the surface of MH and SDA and then dried. Five-millimeters diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the extract was delivered into the wells. The plates were then incubated for 18 h at 35°C. Antimicrobial activity was determined by comparing the minimal inhibition concentration (MIC) values (µg/mL) against the test organism. Ampicillin (10 µg), streptomycin (10 µg), and fluconazole (5 µg) were used as standard drugs and were solved with DMSO. DMSO was therefore used as a vehicle control.

2.7. Cell Culture

Human foreskin fibroblast cells were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were grown in EMEM supplemented with 10 % FBS, 2 mM glutamine, 1% antibiotic solution at 37°C.

2.7.1. Determination of H_2O_2 Concentration

Fibroblast cells were seeded into T-25 flasks at a density of 2×10^5 cells per flask. After 24 h, cells were incubated with 10-30 μM H_2O_2 for 5 min to determine the optimum damaging concentration. After incubation, cells were washed, trypsinized, and centrifuged for comet assay.

2.7.2. Determination of Extract Concentration

Cells were pre-incubated with various concentrations of *D. carmelitarum* extract (100, 250, and 500 μg/mL) for 60 min. After that, flasks were washed with PBS, and the cells were incubated with 20 μM H_2O_2 for 5 min. Following incubation, flasks were washed, trypsinized, and centrifuged for comet protocol.

2.7.3. Cell Viability

Trypan blue dye-exclusion test was used for evaluation of cell viability [23]. After treatments and trypsinizations, the cell suspensions were mixed with trypan blue solution and cell viabilities were determined by direct counting of cells in a neubauer chamber under an inverted microscobe (Nikon Eclipse TS100, Tokyo, Japan). A hundred of cells were counted per group and experiment was run in triplicate.

2.7.4. Comet Assay

The alkaline version of comet assay was used for determination of DNA damage [24] with slight modifications. 75 μL of cell suspension was mixed with 75 μL of 1% low melting agarose, and rapidly spread on the slides pre-coated 0.75% normal melting agarose. The slides were covered with coverslips, and agarose layer was allowed to solidify at 4°C for 5 min. After that, slides were steeped into fresh lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, and 1% (v/v) Triton X-100) for 1 h at 4°C. Then, the slides placed into a horizontal gel electrophoresis tank filled with fresh electrophoresis buffer (300 mM NaOH, 10 mM Na_2EDTA, and 1% (v/v) DMSO, pH:13.1). The slides were left for 30 min to allow unwinding of DNA. Electrophoresis was then carried out in the same buffer for 25 min at 1 V/cm, and 300 mA. After electrophoresis, the slides were incubated 15 min in fresh neutralization buffer (0.4 M Tris buffer, pH:7.5), before staining with ethidium bromide (20 μg/mL). For each treatment condition, 100 randomly selected cells from each slide were evaluated visually using a fluorescent microscope (Nikon Eclipse E800, Tokyo, Japan). Selected cells were classified between 0 (non-damaged) and 3 (most damaged) according to tail lengths. All slides were scored with the following formula [23] with a maximum damage possibility of 300:

$$\text{Comet score} = (1 \times n_1) + (2 \times n_2) + (3 \times n_3) \text{ (n: cell number for every score)}$$

The percent reduction of DNA damage by extract was determined by following equation [23]:

$$\%\ \text{Reduction} = (A\text{-}B)/(A\text{-}C) \times 100$$

where A: comet score of cells treatment with only H_2O_2 (positive control), B: comet score of cells with anti-genotoxic treatment (Extract + H_2O_2) and C: comet score of control cells (no treatment).

2.8. Statistical Analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation. Compatibility with normal distribution was determined using the Kolmogorov-Smirnov test. One-Way ANOVA analysis was used to compare differences among the groups. Statistical significance level was considered as $p < 0.05$.

3. Results and Discussion

Oxidative stress which is caused by increasing ROS and/or decreasing antioxidants and is in a close relationship with many pathological situations, such as cancer, heart diseases, and diabetes [25].

Antioxidant activity is therefore an essential feature for human health. It is believed that many of the biological functions may originate from this feature. Phenolics in natural products might prevent humankind against chronic diseases through their antioxidant action. Determination of antioxidant activity of tested natural product is therefore accepted as a starting point for more comprehensive studies [26]. Many *in vitro* analyses are used for determination of antioxidant capacity of herbal extracts and at least using two different methods are recommended [27]. Consequently, the antioxidant properties of *D. carmelitarum* extract were investigated *in vitro* condition by four different analyses (TPC, FRAP, CUPRAC, and DPPH assay) and results were presented in Table 1. In similar with our findings, Cai *et al.* reported that TPC value of aqueous extract of *D. superbus* L. is 6.9 mg GAE/g sample [26], while Gou *et al.* shown that TPC and DPPH inhibition (SC_{50}) values of aqueous extract of *D. superbus* are 24.04 mg GAE/g sample and 0.9 mg/mL, respectively [28]. In another study, SC_{50} value for the free radical scavenging ability of aqueous extract of *D. basuticus* is reported as 2.56±0.18 µg/mL [8]. The antioxidant compounds contained in plant materials play a major role in the scavenging and inhibiting of free radicals [29]. There is a powerful association between phenolic properties and antioxidant activities. We therefore quantified the total levels of phenolic compounds as GAE. Subsequently, we used the RP-HPLC analysis to identify the phenolic compounds that are responsible for the antioxidant properties of extract.

Phenolic acids are made up of two subgroups, known as the hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids, which share a C6-C1 structure. In contrast, hydroxycinnamic acids are aromatic molecules that possess a three-carbon side chain (C6-C3), the most frequently identified of these being caffeic, ferulic, *p*-coumaric, and sinapic acids [30]. Phenolics are compounds with one or more hydroxyl groups (-OH) conjugated to an aromatic ring. They are found in the aerial parts of plants, including flowers, leaves, seeds, fruits, stems, and roots. Particular research interest has recently been directed toward phenolic compounds because of their beneficial biological characteristics, including antioxidant and radical scavenging properties [31].

Table 1. The antioxidant activities of the aqueous extract of *Dianthus carmelitarum*

Test Compounds	TPC[1]	FRAP[2]	CUPRAC[3]	DPPH[4]
Extract	12.6 ± 0.27	238 ± 2.89	641 ± 6.28	1.2246 ± 0.0042
BHT				0.0099 ± 0.0002

[1]Total phenolic content was expressed as mg GAE per gram sample.
[2]FRAP value was expressed as µM TE per gram sample.
[3]CUPRAC value was expressed as µM TE per gram sample.
[4]Concentration of test sample (mg/mL) required to produce 50% inhibition of the DPPH radical.

The hydroxyl groups in the meta-positions of carboxyl groups (-COOH) are capable of causing greater antioxidant activity compared to the ortho- or para- positions because the electron-pushing characteristic exhibited by the carboxyl group enhances the H-donating capacity of hydroxyl groups. Ortho- substitution on the part of the -OH group with electron-donating compounds, such as methoxy groups ($-OCH_3$) is also capable of enhancing antioxidant activity [32]. For instance, the -CH=CH-COOH groups found in cinnamic acid exhibit a higher H-donating ability and therefore antioxidant properties than the -COOH groups found in benzoic acids by stabilizing the radical through resonance of -C=C- [33]. Phenolic compounds are capable of halting chain oxidation reactions through a range of mechanisms, including the donation of hydrogen atoms or chelation of metal ions. This therefore enables them to behave as metal chelators, antioxidants, reducing agents, or single oxygen and H_2O_2 decomposers. These highly significant plant metabolites also exhibit antioxidant activities [34]. Our antioxidant activity results as soon as were similar with other studies, the differences may have originated from the plant species, phenolic compounds which have important functions in plants, type of extraction method, geographic region, harvest season, and post-harvesting conditions.

Several reports describe the use of HPLC with DAD for characterization and quantification of phenolic composition. It is known that HPLC presents higher robustness, reproducibility, and sensitivity, as well as an interface easily coupled to a great range of detectors [35]. Therefore HPLC-DAD system was preferred for phytochemical analysis in this study and thirteen phenolic compounds were determined

in *D. carmelitarum* (Table 2). The chromatogram of phenolic standards and the extract were shown in Figure 1 and 2, respectively. The developed and validated HPLC method was assessed by different validation parameters as mention above. According to results, the linearity from validation parameters was found to be fine with correlations coefficients between 0.9930 and 0.9997. The detection and quantification limits of each phenolic compounds were remarked in Table 2. Calculated LOD and LOQ values are indicated that applied concentrations ranges (2.5-50 µg/mL) is relevant to study by the method. The recovery of the method was appropriate in terms of the values ranges 98.93-103.86 % considered acceptable values range 95-105 % [36]. The low precision values of %RSD of retention times and % peak areas were pointed out the validity of the method within the acceptable range RSD ≤ 2% (Table 3). All standarts peaks were completely separated through the HPLC conditions. The situation was indicated the method selectivity.

The results of previous researches show that *Dianthus* genus is rich in polyphenolic compounds, such as flavonoids, anthocyanins, coumarins, and anthraquinones [4]. Lamula and Ashafa reported that the aqueous extract of *D. basuticus* has some functional compounds, such as alkaloids, tannins, saponins, and cardiac glycosides. Phlobatannin, flavonoids, steroids, and terpenoids are not detected in the same extract [7]. Also, Ding *et al.* isolated some compounds, such as benzoic acid, kaempferol, quercetrin, 3,5,7-trihydroxy-3',5'-dimethoxyflavone, and trans-*p*-coumaric acid from the ethyl acetate fraction of ethanol extract of *D. superbus* [13]. There was not a fully overlapping between our phenolic composition results and literature data. This situation may have arisen from the plant species and number and kind of used standards. We think that the phenolic composition of *D. carmelitarum* might reveal with further standard compounds in the future investigations.

Especially for a long time, investigations of antioxidant property of natural products have focused on less volatile compounds, such as polyphenolic compounds. However, in recent years strong antioxidant property of volatile compounds are reported. Volatile compounds have therefore begun to arouse much more interest due to their potential antioxidant and antimicrobial activities [37]. SPME which is a free-solvent technique for sample preparation that can be easily coupled to gas chromatography, simplifies the analytical process, is fast, cheap, very useful for volatile and semi-volatile analytes, and suitable for the direct analysis of solid samples [38]. We therefore preferred SPME-GC-FID/MS method for determination of volatile compounds in *D. carmelitarum* and results of volatile analysis were presented in Table 4. Among the identified components were monoterpene hydrocarbons (28.93 %), oxygenated monoterpenes (15 %), aldehydes (28.6 %), hydrocarbones (21.8 %), and other compounds (5.4 %). Among the identified components, monoterpene hydrocarbons are indicated as major volatiles. In a previous study, phenyl ethyl alcohol, eugenol, hexyl benzoate, hexenyl benzoate (z), benzyl benzoate, benzoin, nootkatone, benzyl salyicylate, m-cresyl phenyl acetate, hexadecanoic acid, and eicosene are showed in the composition of *D. caryophyllus* L [39]. Also, Casiglia et al. demonstrated the presence of 66 components which amounted to 93.6% of the oil in the *D. rupicola* and high content of thymol and carvacrol derivatives is shown for the first time in the *Dianthus* genus. Among the idenfied components, terpenes are indicated as major volatiles [5].

The composition of the volatile components we have studied is different from that of volatile components from other *Dianthus* species. In fact, the main components of the *D. superbus* L. oil were 6,10,14-trimethyl-2-pentadecanone (hexahydrofarnesyl acetone, 28.39%), cis-phytol (6.80%), geranyl acetone (4.65%), n-hexanol (4.32%), and farnesyl acetone (3.01%) [5], while the most abundant components of the essential oil of *D. caryophyllus* L. are phenyl ethyl alcohol, eugenol, and hexyl benzoate [41]. Floral scent of seven *Dianthus* species analysed by GC-MS is dominated by different compounds [42]. *Dianthus sylvestris* Wulfen scent is characterised by a great amount of methylbenzoate (85.7%), a compound that is also the main component (42.1%) in *Dianthus arenarius* L. that contains two other benzenoids esters: methyl salicylate (14.5%) and dimethyl salicylate (8.9%).

Table 2. Limit of detection (LOD) and limit of quantification (LOQ) values and quantities of phenolic compounds in the extract

	Phenolic compounds	Standard Curves	Correlation coefficient (r)	LOD (µg/mL)	LOQ (µg/mL)	Amount (mg/100 g)
1	Gallic acid	y=46685x-33209	0.9935	0.03	0.12	20.25±1.11
2	Protocatechuic acid	y=39214x-27944	0.9984	0.04	0.11	5.41±0.24
3	Protocatechuic aldehyde	y=46438x+6147.1	0.9968	0.03	0.09	5.06±0.19
4	*p*-OH-Benzoic acid	y=39080x-10794	0.9974	0.02	0.07	13.71±0.58
5	Chlorogenic acid	y=9208.8x-399.4	0.9930	0.12	0.35	26.03±1.33
6	Vanillic acid	y=49369x-63110	0.9980	0.02	0.08	6.07±0.23
7	Caffeic acid	y=27764x-9126.7	0.9973	0.05	0.15	14.24±0.60
8	Vanillin	y=38753x-1671.6	0.9976	0.02	0.07	7.38±0.39
9	Syringaldehyde	y=13655x-2503.5	0.9982	0.11	0.34	30.3±1.21
10	*p*-Coumaric acid	y=53747x-39534	0.9955	0.03	0.11	12.1±0.51
11	Ferulic acid	y=81674x-55584	0.9975	0.06	0.19	4.49±0.17
12	Sinapic acid	y=63367x-44638	0.9997	0.12	0.38	13.22±0.62
13	Benzoic acid	y=6422x-11532	0.9967	0.28	0.86	15.36±0.91

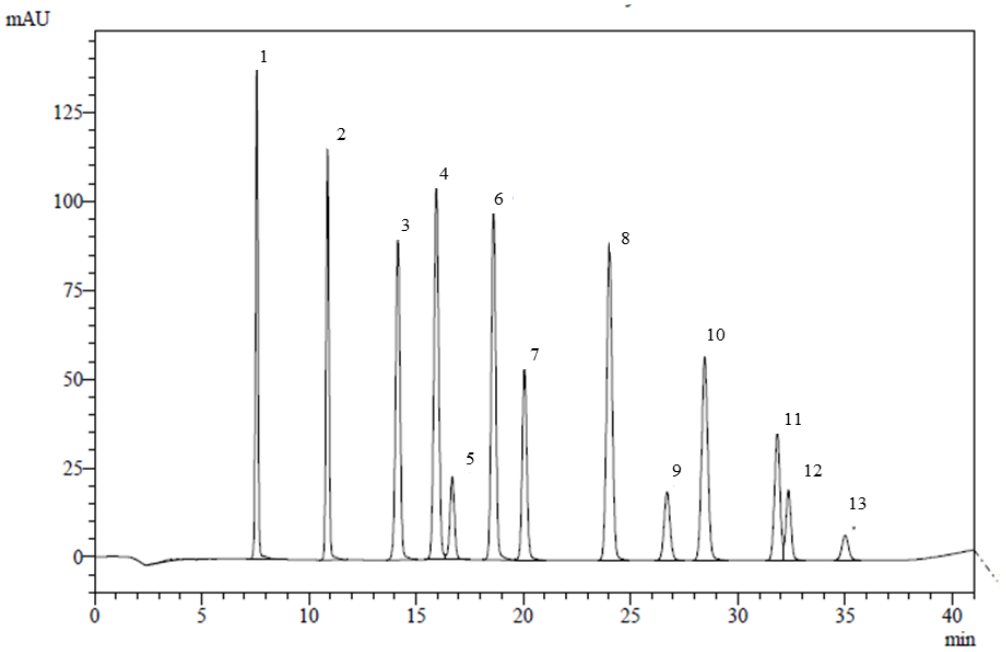

Figure 1. RP-HPLC chromatogram of phenolic standards searched in *D. carmelitarum* sample. Peak identification: (1) gallic acid, (2) *proto*-catechuic acid, (3) *proto*-catechuic aldehyde, (4) *p*-hydroxy benzoic acid, (5) chlorogenic acid, (6) vanillic acid, (7) caffeic acid, (8) vanillin, (9) syringaldehyde, (10)) *p*-coumaric acid, (11) ferulic acid, (12) sinapic acid, (13) benzoic acid.

Table 3. Precision and recovery values of phenolic compounds of the extract

	Phenolic Compounds	RT Precision (% RSD)		% Area Precision (% RSD)		Recovery % (Mean ± SD)		
		Intra day	Inter day	Intra day	Inter day	5 µg/mL	25 µg/mL	50 µg/mL
1	Gallic acid	0.13	0.23	0.13	0.41	101.8±1.19	98.9±2.42	103.8±1.66
2	Proto-catechuic acid	0.16	0.75	0.48	0.63	100.7±2.5	101.6±1.96	103.8±0.65
3	Proto-catechuic aldehyde	0.19	0.38	0.59	0.46	102.0±1.23	102.1±2.08	101.9±1.32
4	*p*-OH Benzoic acid	0.12	0.88	0.29	0.66	101.8±1.27	99.8±1.48	101.1±0.40
5	Chlorogenic acid	0.23	0.48	0.72	0.81	101.5±0.80	103.0±2.05	101.3±0.87
6	Vanillic acid	0.06	0.43	0.11	0.53	101.7±1.05	103.2±2.56	100.8±0.71
7	Caffeic acid	0.04	0.22	0.55	0.60	101.8±1.16	101.9±1.08	101.6±1.5
8	Vanillin	0.03	0.13	0.31	1.81	103.6±2.21	100.5±0.61	101.9±1.36
9	Syringaldehyde	0.02	0.12	0.92	1.01	100.9±0.37	99.2±2.51	100.9±0.45
10	*p*-Coumaric acid	0.06	0.18	0.22	0.37	101.8±1.36	99.7±1.43	101.4±1.70
11	Ferulic acid	0.04	0.21	0.21	0.88	102.0±1.25	101.9±1.4	101.2±0.35
12	Sinapic acid	0.03	0.27	0.71	1.09	102.2±1.15	103.36±1.11	101.3±1.2
13	Benzoic acid	0.07	0.25	1.18	1.61	101.0±1.4	102.76±2.05	102.6±2.25

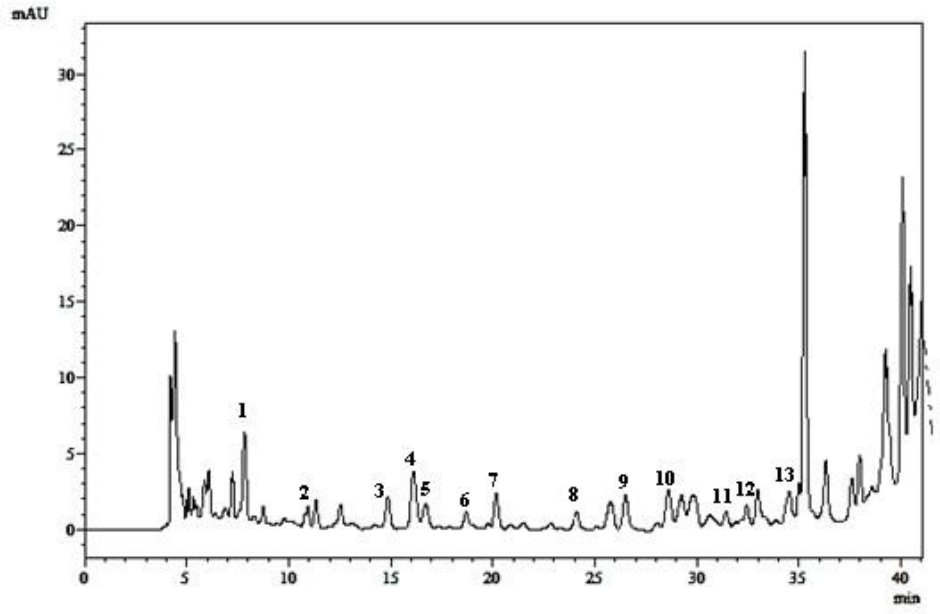

Figure 2. RP-HPLC chromatogram of *Dianthus carmelitarum* sample.

Table 4. Major volatile components of *D. carmelitarum* based on SPME-GC-FID/MS analysis

Identified Compound	%Area[a]	Retention Index[b]
Monoterpene hydrocarbons		
α-Thujene	20.5	930
Camphene	6.7	952
Sabinene	0.71	976
α-Terpinolene	1.02	1095
Total	**28.93**	
Oxygenated monoterpenes		
Pinocarvone	15	1160
Total	**15**	
Aldehydes		
Undecanal	18.6	1300
(E,E)-2,4-Decadienal	10	1315
Total	**28.6**	
Others		
Cadina-1(2),4-diene	5.4	1532
Total	**5.4**	
Hydrocarbons		
Tricosane	21.8	2300
Total	**21.8**	
Total identified	**99.73**	

[a] %Area obtained by FID peak-area normalization.
[b] RI calculated from retention times relative to that of n-alkanes (C6-C30) on the nonpolar Restek Rxi-5MS column.

D. armeria L. is characterised by (Z)-3-hexenyl-1-acetate (29.8%), limonene (11.6%), and n-nonanal (11.0%). Also *D. deltoides* L. shows (Z)-3-hexenyl-1-acetate (27.1%) as the main component with benzaldehyde (15%) and benzeneacetaldehyde (10.6%). n-nonanal (14.7%) is also the most abundant volatile component of the scent of *D. barbatus* L. in which b-caryophyllene (10.3%) is the other most abundant component. An appreciable caryophyllene content (18.5% and 23.5%) is detected in *D. monspessulanus* L. and *D. superbus* L. which, however, are characterised by the high amount of (Z)-b-ocimene (52.4% and 49.8%, respectively). The chemical composition of volatile compounds can change based on several parameters, such as plant species, climatic, seasonal, and geographic conditions, harvest period, and extraction method. We think that differences between our results and literature data may arise from these parameters.

In recent years, problems relating to the implementation of traditional antibiotics, such as antimicrobial resistance, side effects, and high costs, have increased and natural products are considered as alternative agents in order to replace conventional antibiotics [12]. The antimicrobial activity of the extract was evaluated using agar well diffusion method and results were presented in Table 5.

Table 5. Screening for antimicrobial activity of aqueous extract of *D. carmelitarum*

Tested Compounds	Microorganisms and Minimal Inhibition Concentration (µg/mL)								
	Ec	**Yp**	**Pa**	**Sa**	**Ef**	**Bc**	**Ms**	**Ca**	**Sc**
Extract	-	125	250	250	-	-	62.5	-	-
Ampicillin	10	10	>128	35	10	15			
Streptomycin							4		
Fluconazole								>8	>8

Ec: *E. coli*, Yp: *Y. pseudotuberculosis*, Pa: *P. aeruginosa*, Sa: *S. aureus*, Ef: *E. faecalis*, Bc: *B. cereus*, Ms: *M. smegmatis*, Ca: *C. albicans*, Sc: *S. cerevisiae*, (-): no activity of test concentrations (10 000 µg/mL).

D. carmelitarum extract especially exhibited moderate antibacterial activity against *M. smegmatis* and *Y. pseudotuberculosis*, while antifungal activity was not observed against neither *C. albicans* nor *S. cerevisiae*. In previous reports, ethanolic extract of *D. coryophyllum* shows moderate antibacterial effect against *E. coli*, *B. subtilis*, *S. aureus*, *S. epidermidis*, and *P. aeruginosa* and antifungal effect against *A. niger* and *C. albicans* [6], while Lamula and Ashafa reported that aqueous extract of *D. basuticus* shows moderate antibacterial effect against *B. pumilis*, *E. coli*, *S. aureus* and antifungal effect against *C. rugosa*,

C. albicans, and *T. mucoides* [7]. We think that the antibacterial effect of *D.carmelitarum* against *M. smegmatis* and *Y. pseudotuberculosis* now needs to be investigated *in vivo* condition.

The comet assay is frequently used for detection of DNA damage in cellular level due to its cheapness, easiness and more sensitivite than the other assay [23, 24]. Besides, it has been considered as a favorable assay for evaluation the capacity of phytochemicals to protect cells against genotoxic agents [43]. So, we have preferred to determine DNA damage using comet assay in this study. Many oxidant agents, such as methyl methanesulfonate, H_2O_2, ferrous sulfate, and tert-butyl hydroperoxide are used as a DNA damage inducer in antigenotoxicity studies [23,44,45] and H_2O_2 was preferred to generate oxidative DNA damage in fibroblasts in this study. Hydrogen peroxide is a hydrophobic molecule, it can therefore diffuse into the cytoplasm quite easily and can be rapidly transformed into hydroxyl radicals by the Haber-Weiss or Fenton reaction. Hydroxyl radical is the most detrimental type of ROS and it can induce various types of DNA damage, such as strand breaks, alkali-labile sites, oxidized purines and pyrimidines [23]. In this study, comet scoring was performed using a scale of 0 (no damage) to 3 (most damage) with visual analysis between 10 and 30 µM H_2O_2 damaging concentrations. Cell viabilities were measured using trypan blue dye-exclusion method and were over 98% in all groups. Significant increases in DNA damage were determined with increasing concentrations of H_2O_2 (10, and 20 µM; $p < 0.0001$ for both; Figure 3). Maximum comet score would be 300 according to this scale, and the H_2O_2 concentration was therefore selected as 20 µM. Excessively long tails scored at 4 were not included in this study, so 30 µM H_2O_2 were not preferred as an optimum concentration of H_2O_2. Previous reports demonstrate the damaging concentration of H_2O_2 in fibroblast cells changes between 10 and 100 µM. So, our used both concentration of H_2O_2 (20 µM) and incubation time (5 min) were compatible with previous literature [23,46,47].

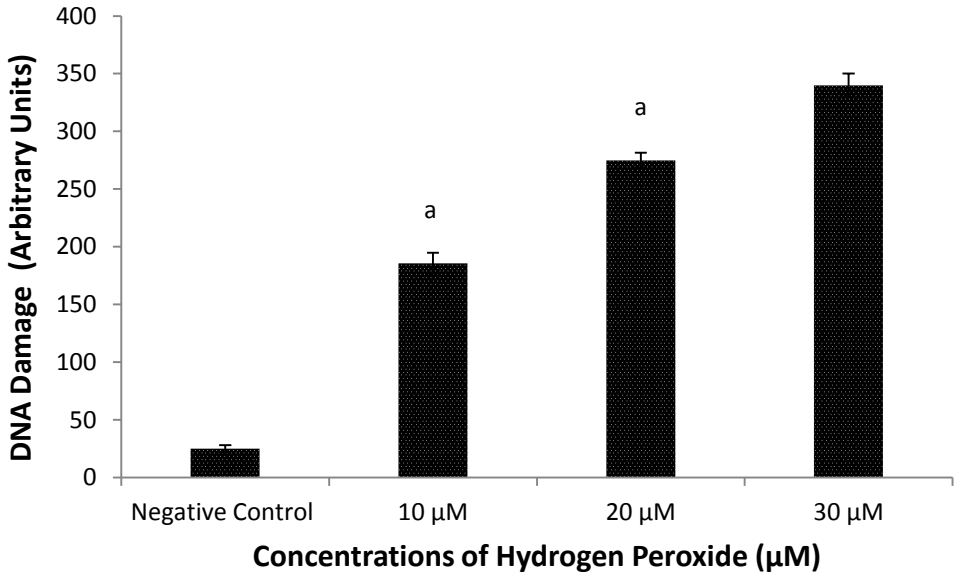

Figure 3. DNA damage of human fibroblasts exposed to hydrogen peroxide. Human fibroblasts were incubated for 5 min with different concentrations of hydrogen peroxide. Results are mean ± SD, (n = 3). [a]$p < 0.0001$ compared to negative control.

In this study, *D. carmelitarum* extract, rich in phenolic and volatile compounds, was investigated in terms of its protective effect on H_2O_2-induced DNA damage in fibroblast cells. The pre-treatment time (1 h) of fibroblast cells with the extract was established according to similar studies about antigenotoxic effect of natural products [21,32,33]. Pre-treatment with different concentrations of the extract significantly protected the cells against H_2O_2-induced DNA damage in a concentration dependent manner ($p<0.0001$) (Figure 4). The percentage reductions of H_2O_2-induced DNA damage were 31%, 47%, and 65% for the concentrations of 100, 250, and 500 µg/mL extract, respectively. Due to pro-oxidant effect of the extract at greater than 500 µg/mL concentration on fibroblast cells, 500 µg/mL concentration was selected as the highest concentration following the preliminary tests. In our study, *D. carmelitarum* extract did not return H_2O_2-induced DNA damage to negative control levels. This in part, may be due to

the pre-incubation time of 1 hours being too short or type of treatment. We think that the antigenotoxic effect of *D. carmelitarum* now needs to be investigated both *in vitro* condition with different treatment types (post or simultaneous) and *in vivo* condition.

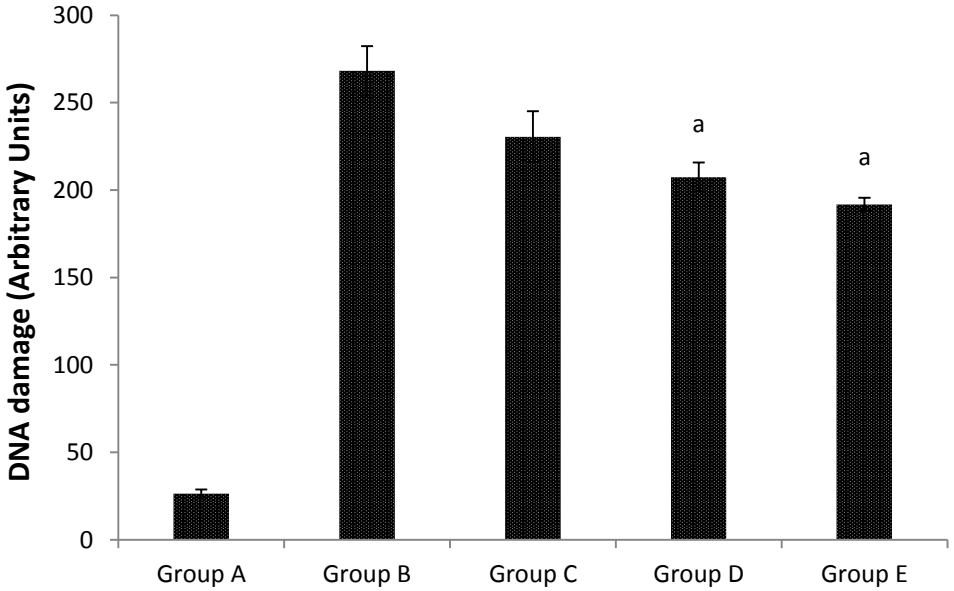

Figure 4. Effect of 1-h pretreatment of the extract on H_2O_2-induced DNA damage in fibroblast cells. Groups: A: negative control; B: positive control (20 µM H_2O_2 alone); C: 100 µg/mL extract + 20 µM H_2O_2; D: 250 µg/mL extract + 20 µM H_2O_2; E: 500 µg/mL extract + 20 µM H_2O_2. Results are mean ± SD, (n = 3). $^{a}p < 0.0001$ compared to positive control.

Many plants contain antioxidant components, such as polyphenolic and volatile compounds and these compounds preserve cells against the detrimental effects of ROS [9]. The antioxidant property of these compounds is attributed to their ability to donate electrons to ROS, chelating metal ions, and stimulate antioxidant enzymes [48]. There are many studies about antigenotoxic activity of these compounds. Ferk *et al.* shown that gallic acid has dose dependent protective effect on H_2O_2-induced DNA damage in human lymphocytes [49], while Cinkilic *et al.* reported that chlorogenic acid decreases X-ray irradiation induced DNA damage and provides a significant radioprotective effect in human lymphocytes [50]. Archana *et al.* reported that thymol reduces radiation-induced DNA damage and apoptosis in Chinese hamster lung fibroblast cells through the free radical scavenging property [51], while Aristatile *et al.* demonstrated that carvacrol prevents human lymphocytes against UVB irradiation-induced DNA and lipid damage [52]. In this study, some of these compounds were determined in the extract and the antigenotoxic effect of *D. carmelitarum* extract may arise from the above mentioned compounds.

To our knowledge, this is the first publication about pytochemical composition, antioxidant, antimicrobial, and antigenotoxic effects of *D. carmelitarum*. Further studies are required for the isolation and identification of individual phenolic and volatile compounds in the extracts. Also, the phytochemical studies together with biological activity investigations are essential for complete understanding of the medicinal applications.

Acknowledgments

The authors thank Resat Aliyazicioglu for his assistance of plant collection. Sıla Ozlem Sener and Merve Badem would like to acknowledge the scholarship during their postgraduate program provided by the Turkish Scientific and Technical Research Council (TUBITAK).

References

[1] A. Obmann, I. Werner, A. Presser, M. Zehl, Z. Swoboda, S. Purevsuren, S. Narantuya, C. Kletter, and S. Glasl (2011). Flavonoid C- and O-glycosides from the Mongolian medicinal plant *Dianthus versicolor* Fisch, *Carbohydr. Res*. **346**, 1868-1875.

[2] H. Reeve (1967). *Dianthus* L. In: Flora of Turkey and the East Aegean Islands, ed: P.H. Davis, Edinburgh University Press, Edinburgh, pp. 99-131.

[3] E. Hamzaoglu, M. Koc, I. Buyuk, A. Aksoy, and S. Soydam Aydin (2015). Presence of *Dianthus roseoluteus* Velen. (*Caryophyllaceae*) in Turkey and a new species: *Dianthus macroflorus* Hamzaoglu, *Syst. Botany.* **40**, 208-213.

[4] J.Q. Yu, Y. Yin, J.C. Lei, X.Q. Zhang, W. Chen, C.L. Ding, S. Wu, X.Y. He, Y.W. Liu, and G.L. Zou (2012). Activation of apoptosis by ethyl acetate fraction of ethanol extract of *Dianthus superbus* in HepG2 cell line, *Cancer Epidemiol*. **36**, e40-e45.

[5] S. Casiglia, M. Bruno, and F. Senatore (2014). Volatile constituents of *Dianthus rupicola* Biv. from Sicily: Activity against microorganisms affecting cellulosic objects, *Nat. Prod. Res.* **28**, 1739-1746.

[6] O. Erturk (2006). Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants, *Biologia* **61**, 275-278.

[7] S.Q.N. Lamula and A.O.T. Ashafa (2014). Antimicrobial and cytotoxic potential of *Dianthus basuticus* used in Basotho traditional practice, *Bangladesh J. Pharmacol.* **9**, 105-111.

[8] M.I. Kazeem and A.O.T. Ashafa (2015). *In-vitro* antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts, *J. Herb. Med.* **5**, 158-164.

[9] H.R. Park, E. Park, A.R. Rim, K.I. Jeon, J.H. Hwang, and S.C. Lee (2006). Antioxidant activity of extracts from *Acanthopanax senticosus, Afr. J. Biotechnol.* **5**, 2388-2396.

[10] D.D. Orhan, B. Ozcelik, S. Hosbas, and M. Vural (2012). Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi, *Turk. J. Biol.* **36**, 672-686.

[11] D.E. Djeussi, J.A.K. Noumedem, J.A. Seukep, A.G. Fankam, I.K. Voukeng, S.B. Tankeo, A.H.L. Nkuete, and V. Kuete (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria, *BMC Complement. Alternat. Med.* **13**, 164.

[12] V. Aleksic and P. Knezevic (2014). Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L., *Microbiol. Res.* **169**, 240-254.

[13] C. Ding, W. Zhang, J. Li, J. Lei, and J. Yu (2013). Cytotoxic constituents of ethyl acetate fraction from *Dianthus superbus, Nat. Prod. Res.* **27**, 1691-1694.

[14] A.O.T. Ashafa and M.I. Kazeem (2015). Toxicopathological evaluation of hydroethanol extract of *Dianthus basuticus* in wistar rats, *Evid. Based Complement. Alternat. Med.* Article ID: **348519**, Doi: 10.1155/2015/348519.

[15] K. Slinkard and V. L. Singleton (1977). Total phenol analyses: automation and comparison with manual methods, *Am. J. Enol. Viticult.* **28**, 49-55.

[16] I.F.F. Benzie and J.J. Strain (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, *Method. Enzymol.* **299**, 15-27.

[17] M.S. Blois (1958). Antioxidant determinations by the use of a stable free radical, *Nature* **181**, 1199-1200.

[18] R. Apak, K. Guclu, M. Ozyurek, and S.E. Karademir (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, *J. Agric. Food Chem.* **52,** 7970-7981.

[19] International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures, ICH-Q2A, Geneva, 1995.

[20] R. Aliyazicioglu, G. Tosun, N. Yayli, and O.E. Eyupoglu (2015). Characterisation of volatile compounds by SPME and GC-FID/MS of capers (*Capparis spinosa* L.), *Afr. J Agric. Res.* **10**, 2213-2217.

[21] C. Bicchi, C. Cordero, E. Liberto, B. Sgorbini, and P. Rubiolo (2008). Headspace sampling of the volatile fraction of vegetable matrices, *J. Chromatogr. A.* **1184**, 220-233.

[22] I. Ahmad, Z. Mehmood, and F. Mohammad (1998). Screening of some Indian medicinal plants for their antimicrobial properties, *J. Ethnopharmacol.* **62**, 183-193.

[23] Y. Aliyazicioglu, S. Demir, I. Turan, T. N. Cakiroglu, I. Akalin, O. Deger, and A. Bedir (2011). Preventive and protective effects of Turkish propolis on H_2O_2-induced DNA damage in foreskin fibroblast cell lines, *Acta Biol. Hung.* **62**, 388-396.

[24] N.P. Singh, M.T. McCoy, R.R. Tice, and E.L. Schneider (1988). A simple technique for quantitation of low levels of DNA damage in individual cells, *Exp. Cell Res.* **175**, 184-191.

[25] C. Kaur and H.C. Kapoor (2001). Antioxidants in fruits and vegetables-the millennium's health, *Int. J. Food. Sci. Tech.* **36**, 703-725.

[26] Y. Cai, Q. Luo, M. Sun, and H. Corke (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, *Life Sci*. **74**, 2157-2184.

[27] J.H. Lee, S.J. Lee, S. Park, H.K. Kim, W.Y. Jeong, J.Y. Choi, N.J. Sung, W.S. Lee, C.S. Lim, G..S. Kim, and S.C. Shin (2011). Characterisation of flavonoids in *Orostachys japonicus* A. Berger using HPLC-MS/MS: Contribution to the overall antioxidant effect, *Food Chem.* **124**, 1627-1633.

[28] J. Gou, Y. Zou, and J. Ahn (2011). Enhancement of antioxidant and antimicrobial activities of *Dianthus superbus*, *Polygonum aviculare*, *Sophora flavescens*, and *Lygodium japonicum* by pressure-assisted water extraction, *Food Sci. Biotechnol.* **20**, 283-287.

[29] M.Bener, M. Özyürek, K. Güçlü and R Apak (2016). Optimization of microwave-assisted extraction of curcumin from *Curcuma longa* L. (Turmeric) and evaluation of antioxidant activity in multi-test systems, *Rec.Nat. Prod.* **10,** 542-554.

[30] L. Bravo (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance, *Nutr. Rev.* **56,** 317-333.

[31] P. Kalın, İ. Gülçin and A.C. Gören (2015). Antioxidant activity and polyphenol content of Cranberries (*Vaccinium macrocarpon*), *Rec.Nat. Prod.* **9,** 496-502.

[32] M. Oyaizu (1986). Studies on product of browning reaction prepared from glucose amine, *Jpn. J. Nutr.* **44,** 307-315.

[33] H. Chen, Y. Zhou, Y. Shao, and F. Chen (2016). Free phenolic acids in shanxi aged vinegar: changes during aging and synergistic antioxidant activities, *Int. J. Food Prop.* **19,** 1183-1193.

[34] I. Gulcin, E. Kirecci, E. Akkemik, F. Topal, and O. Hisar (2010). Antioxidant and antimicrobial activities of an aquatic plant: Duckweed (Lemna Minor L.), *Turk. J. Biol.* **34,** 175-188.

[35] M.T.S. Trevisan, R. A. Marques , M. G.V. Silva,D. Scherer, R. Haubner, C. M. Ulrich and R.W. Owen (2016). Composition of essential oils and ethanol extracts of the leaves of Lippia species: Identification, quantitation and antioxidant capacity, *Rec.Nat. Prod.* **10,** 485-496.

[36] I. Rosenthal, E. Wolfram, S. Peter, and B. Meier (2014). Validated method for the analysis of frangulins A and B and glucofrangulins A and B using HPLC and UHPLC, *J. Nat. Prod.* **77,** 489-496.

[37] H.W. Jang, J.K. Moon, and T. Shibamoto (2015). Analysis and antioxidant activity of extracts from broccoli (*Brassica oleracea* L.) sprouts, *J. Agric. Food Chem.* **63**, 1169-1174.

[38] J.D. Carrillo and M.T. Tena (2005). Determination of volatile compounds in antioxidant rosemary extracts by solid-phase microextraction and gas chromatography, *Anal. Lett.* **38**, 1193-1212.

[39] A.H. El-Ghorab, M.H. Mahgoub, and M. Bekheta (2006). Effect of some bioregulators on the chemical composition of essential oil and its antioxidant activity of Egyptian carnation (*Dianthus caryophyllus* L.), *J. Essent. Oil Bear. Plant* **9**, 214-222.

[40] J. Yu, Z. Liao, X. Cai, and G. Zou (2008). Analysis of essential oil chemical compositions of *Dianthus superbus* with GC-MS, *Chin. Hosp. Pharm. J.* **28,** 157-158.

[41] A.H. El-Ghorab, M.H. Mahgoub, and M. Bekheta (2006). Effect of some bioregulators on the chemical composition of essential oil and its antioxidant activity of Egyptian Carnation (*Dianthus caryophyllus* L.), *JEOBP* **9,** 214-222.

[42] A. Jurgens, T. Witt, and G. Gottsberger (2003). Flower scent composition in *Dianthus* and *Saponaria* species (*Caryophyllaceae*) and its relevance for pollination biology and taxonomy, *Biochem. Syst. Ecol.* **31,** 345-357.

[43] A.R. Collins (2005). Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols, *Am. J. Clin. Nutr.* **81**, 261S-267S.

[44] C.C. Munari, J.M. Alves, J.K. Bastos, and D.C. Tavares (2009). Evaluation of the genotoxic and antigenotoxic potential of *Baccharis dracunculifolia* extract on V79 cells by the comet assay, *J. Appl. Toxicol.* **30**, 22-28.

[45] I. Turan, O. Deger, Y. Aliyazicioglu, S. Demir, K. Kilinc, and A. Sumer (2015). Effects of Turkish propolis on expression of hOGG-1 and NEIL-1, *Turk. J. Med. Sci.* **45**, 804-811.

[46] S. Teramoto, T. Tomita, H. Matsui, E. Ohga, T. Matsuse, and Y. Ouchi (1999). Hydrogen peroxide induced apoptosis and necrosis in human lung fibroblasts: Protective roles of glutathione, *Jpn. J. Pharmacol.* **79**, 33-40.

[47] X. Chen, H. Nishida, and T. Konishi (2003). Baicalin promoted the repair of DNA single strand breakage caused by H_2O_2 in cultured NIH3T3 fibroblasts, *Biol. Pharm. Bull.* **26**, 282-284.

[48] C.A. Rice-Evans, N.T. Miller, and G. Paganga (1997). Antioxidant properties of phenolic compounds, *Trends Plant. Sci.* **4**, 304-309.

[49] F. Ferk, A. Chakraborty, W. Jager, M. Kundi, J. Bichler, M. Misik, K.H. Wagner, B. Grasl-Kraupp, S. Sagmeister, G. Haidinger, C. Hoelzl, A. Nersesyan, M. Dusinska, T. Simic, and S. Knasmüller (2011). Potent protection of gallic acid against DNA oxidation: results of human and animal experiments, *Mutat. Res.* **715**, 61-71.

[50] N. Cinkilic, S.K. Cetintas, T. Zorlu, O. Vatan, D. Yilmaz, T. Cavas, S. Tunc, L. Ozkan, and R. Bilaloglu (2013). Radioprotection by two phenolic compounds: chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes *in vitro*, *Food Chem. Toxicol*. **53**, 359-363.

[51] P.R. Archana, B.N. Rao, and B.S. Satish Rao (2011). Modulation of gamma ray-inducedg effect by thymol, a monoterpene phenol derivative of cymene, *Integr. Cancer Ther.* **10**, 374-383.

[52] B. Aristatile, K.S. Al-Numair, A.H. Al-Assaf, C. Veeramani, and K.V. Pugalendi (2015). Protective effect of carvacrol on oxidative stress and cellular DNA damage induced by UVB irradiation in human peripheral lymphocytes, *J. Biochem. Mol. Toxic.* **29**, 497-507.