Research Article

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Antioxidant, DNA–damage protection and anti–cancer properties of *n*–butanol extract of the endemic *Perralderia coronopifolia*

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Article Info

Abstract

This study was designed to evaluate *in vitro* the total phenolic, flavonoid content, antioxidant activity, oxidative DNA-damage protection and anti-cancer activity of *n*-butanol extract from *Perralderia coronopifolia*, endemic plant. DNA protection capacity was performed using 46966 plasmid DNA against UV-photolysis of H_2O_2-induced oxidative damage. The antiproliferative effects of extract were performed on HeLa and C6 cells. Experimental results showed that extract had convenient number of phenolic and flavonoids. Furthermore, it provided strong antioxidant, reducing power, hydrogen peroxide and higher DPPH· scavenging ability with IC_{50}= 7.02 ± 0.02 μg/mL. As it shown an oxidative plasmid DNA-damage inhibition against UV-photolysis of H_2O_2, an anti-cancer activity at higher concentrations in cells. The data obtained that *P. coronopifolia* extract could be useful in human protection against infection and degenerative illness.

Introduction

Oxidative stress is the most important factor in the stimulation of certain chronic and degenerative illnesses. DNA damage is the important manifestation of long term ROS exposure, thereby inducing a various disorder in humans (Souri et al., 2008). Some findings suggest that DNA damage may be a major factor risk for several pathogenesis and deceases such as neurodegenerative (Lezza et al., 1999) and cardiovascular diseases (Collins et al., 1998), as well as diabetes (Dandona et al., 1996) and cancer (Kumar et al., 2012).

Anti-oxidants from natural or synthetic products provide novel possibilities to protect and treat oxidative damage by inhibiting or scavenging free radicals (Casa-grande et al., 2006). The investigation of several parts of phenolic plants like fruit, vegetable, nut, seed, leaf, root and bark established their potent pharmacological activities and medicinal properties.

The current study report on *P. coronopifolia* (Asteraceae). It is one of the Algerian endemic species which is seemed to be used as a natural therapeutic remedy, grow in the elevated regions in the North-Western of Africa (Algeria, Morocco and Libya) (Boussaha et al., 2015). Considering the traditional therapeutic use of this plant, it is necessary to evaluate its biological and oxidative potentials.

Therefore, the main purpose of this study was to investigate the antioxidant and the anti-cancer pro-

perties of *n*-butanol extract from *P. coronopifolia* using *in vitro* models' systems to know its consumption of total phenolic content and flavonoids. As well as its anti-oxidant activity, reducing power, hydrogen peroxide scavenging, DPPH· scavenging and metal chelating activities. Furthermore, our study reports the inhibition of oxidative 46966 plasmid DNA-damage against UV-photolysis of H_2O_2, and the inhibition of proliferation of different cancer-cell lines.

Materials and Methods

Chemicals

The chemicals utilized in the tests were in analytical grade and purchased from the Sigma–Aldrich and Roche.

Plant material

The plant material (*P. coronopifolia*) was collected in 2011 at an elevated region located around Taghit in the South-West of Algeria. It was authenticated by M Mouhamed Ben Abdelhakem, manager of the wildlife preserving organization of Bechar. A label sample (PCA0511-TAG-ALG-52) was put at the herbarium of VARENBIOMOL Unit, University of Mentouri1, Constantine.

A dried aerial parts (1,400 g) of the plant were mace-rated for 48 hours at room temperature with ethanol-water (80:20, v/v), three often. The rest was concentrated and liquefied in 650 mL of water, after filtration. The resultant mixture was treated by chloroform, ethyl acetate and *n*-butanol, respectively. The organic phases were dried over Na_2SO_4 and filtered by common filter paper and concentrated in vacuum at ambient temperature to get the chloroform (2 g), ethyl acetate (7 g) and *n*-butanol (40 g) extracts.

Antioxidant properties

Determination of total phenolic and flavonoid contents

Total flavonoid was measured by the Folin-Ciocalteus reagent (FCR) according to the method described by Singleton et al. (1999) using gallic acid (GA) as a standard phenolic compound. 100 µL of FCR and 1.6 mL of distilled water were mixed with 20 µL of extract. After 3-8 min, 0.3 mL of Na_2CO_3 was added. The absorbance of sample was read at 765 nm after 30 min of incubation at 37°C. The total phenolic compounds concentration of extract was quantitated as µg of gallic acid equivalent (GAE)/mg plant material with standard deviation.

Total flavonoid content was determined as described in a reference (Wang et al., 2008) using quercetin as standard. 0.5 mL of sample was added to of $AlCl_3$ (2%; 0.5 mL). The absorbance of sample was read at 420 nm lasting 1 hour of incubation. The quercetin was used as a standard. The content of flavonoid compounds in extract was estimated as µg quercetin equivalent (QE)/mg plant material with standard deviation.

Determination of total antioxidant activity

The activity of the *n*-butanol extract was determined spectrophotometrically as mention in the phospho-molybdenum method (Prieto et al., 1999). This assay founded on the potent of reducing Mo(VI) to Mo(V) by the extract analyzed and the resulting of green phos-phate/Mo(V) substances. The extract solution (0.3 mL) was mixed with 2.7 mL of the reagent solution prepared in ammonium molybdate (4 mM), sulfuric acid (0.6 M) and sodium phosphate (28 mM). The sample and standard solutions were left in a water bath at 95°C during 90 min. After cooling the samples to room temperature, the absorbance of the resulting green sample-phospho-molybdenum complex was read at 695 nm. For the blank, just a replacement of sample by 0.3 mL of ethanol. A typical blank solution contained 2.7 mL of reagent solution, after it was left under the similar conditions as sample. The activity was defined as the absorbance of the sample and standard. A higher absorbance value is indicative a higher antioxidant capacity.

Reducing power assay

The reducing power of *n*-butanol extract and standard anti-oxidants was measured spectrophotometrically using the method of Oyaizu (1986). This assay established on the reduction of the Fe^{+3}-ferricyanide to the Fe^{+2}, by the reductant compounds in the mixture. 1 mL of the sample solutions (50-500 µg/mL) were added to 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%; 2.5 mL). The mixtures were placed in a water bath at 50°C pending 20 min. The mixtures were centrifuged at 1000 x g for 20 min after adding of tricholoroacetic acid (10%; 2.5 mL). Finally, the supernatant (2.5 mL) was added to 2.5 mL of distilled water and ferric chloride (0.5 mL; 0.1%). The mixture intensity of the bleu-green color was read at 700 nm. In this assay, the upper absorbance of sample indicates better reducing power under the reaction conditions.

H_2O_2 scavenging activity

H_2O_2 scavenging activity was measured as a slightly modification described by Zhao et al. (2006). 1 mL of sample was mixed with H_2O_2 (0.1 mM; 1 mL), ammonium molybdate (3%; 100 µL), H_2SO_4 (2 M; 10 mL) and KI (1.8 M; 7 mL). Finally, the mixture was titrated using $Na_2S_2O_3$ (5 mM) till disappearing yellow color. The reaction without sample was used like a control. The percentage of scavenging effect was calculated using the formula given bellow:

H_2O_2 scavenging activity, % = $[(V_{control}-V_{sample})/V_{control}]$ x 100

Where, $V_{control}$ is the volume of $Na_2S_2O_3$ and V_{sample} is the

volume of $Na_2S_2O_3$ solution H_2O_2 added to the test samples

DPPH· scavenging assay

DPPH· scavenging assay was performed according to the method in the literature (Braca et al., 2001). 3 mL of a methanol solution of DPPH· were mixed with 1 mL of sample in various concentrations (1-20 µg/mL). After 30 min incubation in the dark at room, absorbance was read at 517 mm. The inhibition percentage was determined using the formula:

Inhibition, % = $(1 - A_1/A_0)$ x 100

Where, A_1 is absorbance of sample and A_0 is absorbance of control

Metal chelating activity

The Fe^{+2} chelating activity of extract and standard antioxidants was measured as a mentioned assay by Dinis et al. (1994). The different concentrations (50-500 µg/mL) of the sample were added separately to 2 mM of $FeCl_2$ (0.05 mL) and left for incubation at room temperature for 5 min. The reaction was started by adding the ferrozine (5 mM; 0.3 mL). After shaking forcefully, the mixture was incubated at room temperature pending 10 min. EDTA was used as a positive control. Absorbance was read at 562 nm. The inhibition percentage of Fe^{2+}-ferrozine complex creation was measured as the next formula:

Chelating capacity, % = $[(A_0-A_1)/A_0]$ x 100

Where, A_0 is the absorbance of the Fe^{2+}-ferrozine complex and A_1 is the absorbance of the test solutions

DNA damage inhibition efficiency

DNA protection capacity of the test plant extract was performed as descript by Russo et al. (2001), using photolyzing 46966 plasmid DNA (extracted from *E. coli*) through UV radiation in the presence of H_2O_2 and the performance of agarose gel electrophoresis with the irradiated DNA. 1 µL aliquot of 46966 plasmid (20 µg/mL) was put in polyethylene microcentrifuge tube, then 50 µg of *n*-butanol extract plant was added to single tube. One tube did not contain the extract was served as an irradiated control (C_R). Then, 4 µL of H_2O_2 (3%) was added into the tube (including C_R). Lastly, tubes were put on the outer face of a UV transilluminator (300 nm) and irradiated at room temperature during 10 min. Individual tube contains 1 µL aliquots of 46966 plasmid DNA was placed also, which performed as the non-irradiated control (CO). Samples were run on agarose gel (1%) and photographed using a Lourmat gel imaging system (Vilber).

Anti-cancer activity

The xCELLigence RTCA-SP (Real Time Cell Analyzer-Single Plate) instrument is a grouping of four parts: An E-Plate 96, a plate station, an analyzer and a computer including RTCA software. It was used to evaluate the antiproliferative effects of the *n*-butanol extract of *P. coronopifolia* on HeLa and C6-cell lines (Demirtas et al., 2009).

HeLa (human cervix carcinoma) and C6 (rat brain tumor) cells were developed in DMEM-HG (Dulbecco's modified eagle's medium) with 10% (v/v) FBS (fetal bovine serum) and 2% penicilin/streptomycin solution, at 37°C in a humidified atmosphere of 5% CO_2.

HeLa and C6-cells were detached from bottom of the culture flask using 10 mL trypsin-EDTA mixture. After detaching, the same volume of culture medium was placed in the flask and mixed thoroughly and put in centrifuge 600 rpm for 5 min (Nüve NF 800, Turkey). 5 mL of medium was mixed carefully with the supernatant. The concentration of cells in this suspension was determined using CEDEX HIRES Cell Counter.

50 µL of the medium was placed to every well of E-Plate 96 and left in the sterile cabinet for 15 min, then incubated for 15 min. 100 µL of the cell suspension (2,5 x 10^4 cells/100 µL) was placed into wells. Exceptionally, 100 µL of 5-florourasil (5-FU) was added as a control in the last wells. The plate incubated in the hood for 30 min in order to adhere cells to the bottom, then attached to xCELLigence RTCA-SP station in the incubator. A measurement during 80 min was started. The extract solutions (50, 100 and 250 µg/mL) in DMSO were injected to the wells and adjusted until 200 µL using culture medium. The control and medium wells did not have any extract solutions. Lastly, the plate was placed in the incubator xCELLigence instrument and measurements were taken over 48 hours.

Statistical analysis

Data were presented as mean ± standard deviation of the mean of three parallel measurements. The analyses of results were performed by using the SPSS 18 Software and evaluated by t-test.

Results

The content of total phenolics and flavonoids

The *n*-butanol extract of *P. coronopifolia* contained high phenolic compounds (444.0 ± 0.9 µg GAE/mg plant material) and flavonoids (400.3 ± 0.5 µg quercetine equivalents/mg plant material).

Total antioxidant activity

The extract has a high antioxidant activity, but it was inferior to those of antioxidant standards (Table I). All samples exhibited a concentration-dependent total antioxidant activity. At 500 µg/mL, *n*-butanol extract exerted a strongest antioxidant activity (3.0) and followed

Table I				
In vitro anti-oxidant activity of *P. coronopifolia* extract				
Sample	50 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL
Total anti-oxidant activity, 695 nm				
n-Butanol extract	0.03 ± 0.01	0.2 ± 0.01	1.5 ± 0.06	3.0 ± 0.1
Ascorbic acid	0.04 ± 0.02	0.3 ± 0.01	1.0 ± 0.07	2.7 ± 0.02
Butylated hydroxyanisole	0.07 ± 0.01	0. 2 ± 0.02	0.7 ± 0.02	1.5 ± 0.06
Reducing power, 700 nm				
n-Butanol extract	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.4 ± 0.01
Ascorbic acid	0.5 ± 0.005	1.1 ± 0.03	3.5 ± 0.2	3.8 ± 0.06
Butylated hydroxyanisole	0.3 ± 0.01	0.6 ± 0.02	1.2 ± 0.1	3.5 ± 0.05
Trolox	0.2 ± 0.01	0.4 ± 0.01	0.9 ± 0.05	1.9 ± 0.1
Hydrogen peroxide scavenging activity, %				
n-Butanol extract	31.7 ± 3.2	38.9 ± 2.7	47.6 ± 0.87	65.6 ± 0.5
Ascorbic acid	17.5 ± 0.8	34.1 ± 2.1	54.0 ± 0.9	66.7 ± 0.6
a-Tocopherol	30.2 ± 0.9	38.1 ± 0.9	47.6 ± 3.6	55.6 ± 0.8
Butylated hydroxyanisole	25.4 ± 3.2	41.3 ± 3.9	50.8 ± 4.1	60.3 ± 3.8
Trolox	31.7 ± 2.1	41.3 ± 0.8	46.0 ± 2.7	50.8 ± 0.6
Metal chelating activity, %				
n-Butanol extract	7. 9 ± 1.3	29.2 ± 0.7	41.7 ± 2.0	50.9 ± 1.2
Ethylenediaminetetraacetic acid	10.2 ± 1.3	21.9 ± 0.7	37.5 ± 3.0	45.9 ± 1.2
Ascorbic acid	6.5 ± 2.7	10.6 ± 0.5	23.2 ± 1.2	40.6 ± 0.8
a-Tocopherol	6.2 ± 1.3	8.9 ± 1.8	20.2 ± 2.1	23.6 ± 3.2
Butylated hydroxyanisole	2.1 ± 0.4	21.1 ± 0.7	36.8 ± 3.7	52.6 ± 1.8

by ascorbic acid (2.7), and BHA (1.5).

Reducing power activity

The *n*-butanol extract showed a stronger reducing power comparable to those of antioxidant standards used as positive controls: Trolox, BHA, α-tocopherol and ascorbic acid (Table I). It increased with increasing concentrations, ($p < 0.05$) significantly.

H_2O_2 scavenging activity

The H_2O_2 scavenging activity of *n*-butanol extract was compared with ascorbic acid, α-tocopherol, BHA and trolox (Table I). It was determined that the hydrogen peroxide scavenging activities of the samples increased depending on the different concentrations. The H_2O_2 scavenging activity of *n*-butanol extract was higher than that of α-tocopherol, BHA and trolox ($p < 0.05$).

DPPH· scavenging activity

The measurements of DPPH· scavenging capacities of *n*-butanol extract and ascorbic acid as standard radical scavenger indicated in the order of ascorbic acid > *n*-butanol with IC_{50} value of 5.0 ± 0.1 and 7.0 ± 0.02 µg/mL, respectively (Figure 1). The activity of extract was close to that of ascorbic acid.
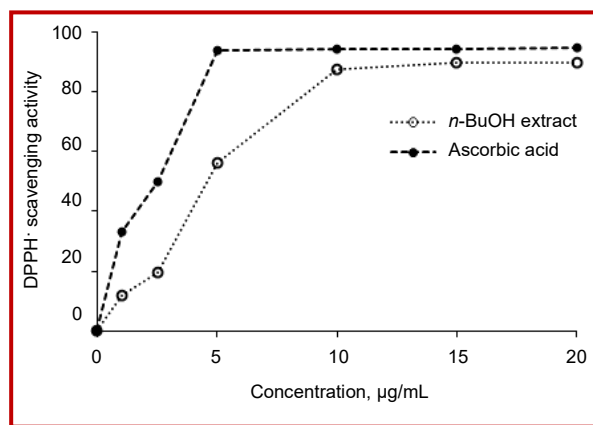

Figure 1: The free radical (DPPH·) scavenging of the *P. coronopifolia n*-butanol extract

Metal chelating activity

The *n*-butanol extract of *P. coronopifolia* was able to chelate Fe^{2+} in a concentration-dependent manner (Table I). At 100 and 250 µg/mL, the metal chelating activity of extract showed higher than that of BHA and ascorbic acid. Except for BHA, the extract exhibited higher chelating activity than standards at 500 µg/mL.

DNA damage inhibition efficiency

Results showed that the extract at a dose of 50 µg was exhibited a completed protection of plasmid DNA. The circular (C) form of untreated DNA was converted into nicked form: Relaxed (R) and/or linear (L), which indicates the plasmid-DNA damaging. While the upon-treatment with *n*-butanol extract of *P. coronopifolia* regained the DNA to its native circular form (Figure 2).
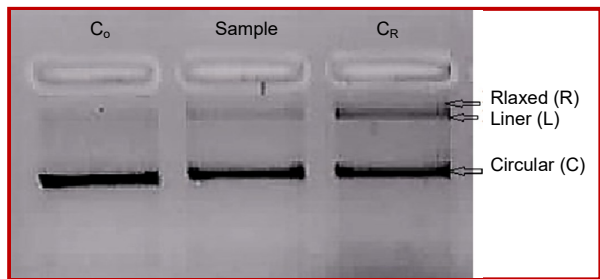
Anti-cancer activity

Figure 2: Effect of anti-cancer activity (50 µg) against oxidative to 46966 plasmid DNA damage caused by UV-photolysis of H_2O_2 (3%, v/v)
C_0 = untreated non-irradiated DNA; Sample = DNA UV-irradiated treated with *n*-butanol extract of *P. coronopifolia*; C_R = untreated UV-irradiated DNA

Figure 3 exhibits the diagram of the anti-cancer test with deviation bars. In the present work as compared with the positive control, the extract revealed diverse profiles at diverse concentrations (250, 100 and 50 µg/mL). As seen in diagram Figure 3A, during the first two hours cells not treated with extracts were observed a normal growth. 24 hours post-treatment, HeLa cells were inactivated by *n*-butanol extract and showed the maxi-mum activity through the concentration of 250 µg/mL. Lower activities were obtained at lower concentrations (100 and 50 µg/mL), nearly same with control during the same period of time.

However, profiles exhibited dissimilarities at various time points, which lower concentrations indicated certain positive inhibition-activities against C6 cells-proliferation during 27 hours post-treatment. As shown in Figure 3C, after a short time they are missing their capacities of inhibition.

Discussion

The antioxidant activity of extract is the result of an existence of polyphenols and flavonoids, which possibly act upon by offering electrons and free radicals. Similar finding was obtained by Barros et al. (2007) and Ozen et al. (2011) on their studies on different extracts. Flavonoid compounds are the most essential plant elements by the reason of their ·OH groups which can offer the antioxidant capacity (Kumar et al., 2008). They
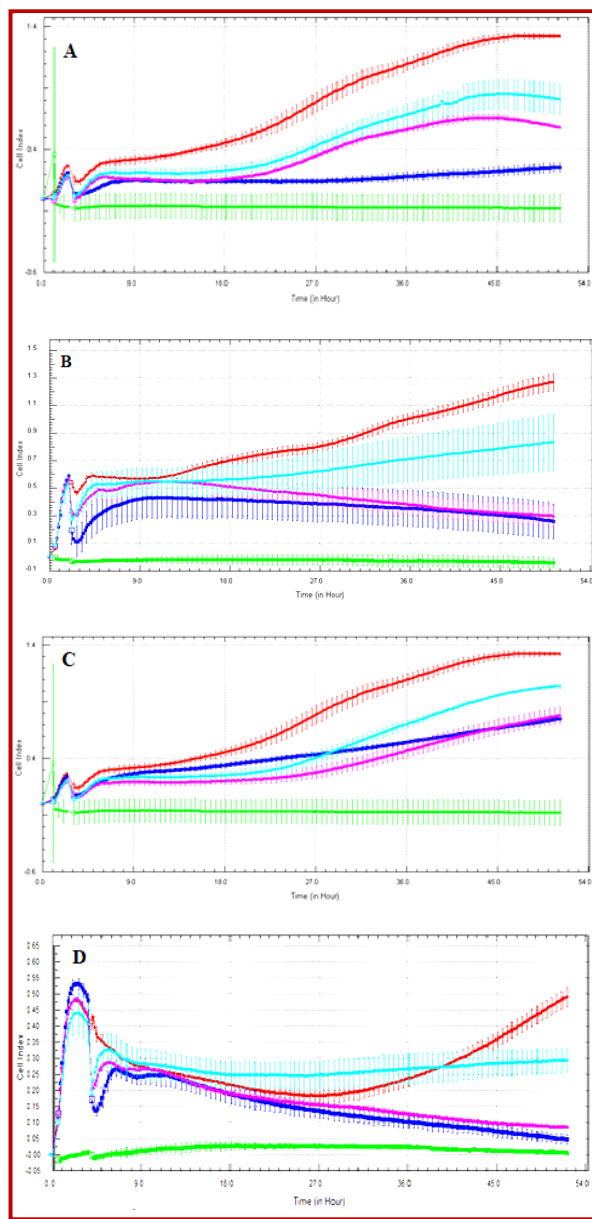

Figure 3: The anti-cancer activity of the *n*-butanol extract of *P. coronopifolia* against HeLa and C6 (2,5 x 10⁴ cell/well) cells line at several extract concentrations (— 50 µg/mL, — 100 µg/mL, — 250 µg/mL, — Control, — Medium).
Profileration assay of the *n*-butanol extract of *P. coronopifolia* (A) and the 5-fluorouracil as a positive control (B) against HeLa cells; Proliferation assay of the *n*-butanol extract of *P. Coronopifolia* (C) and the 5-fluorouracil as a positive control (D) against C6 cells

have been shown to have potent antioxidant activity. The *n*-butanol extract shows that it can act as a radical chain terminator that converts reactive free radicals to stable and non-reactive products. This is the origin of their capability to prevent lipid peroxidation chain reactions by donating a hydrogen atom from their ·OH

groups. In addition, it was reported that phenolic and flavonoid contents are related to antioxidant capacity and have a necessary role in stabilizing for the lipid peroxidation (Koldaş et al., 2015).

The literature reported that the reductive ability of products is commonly related with the existence of reductants like antioxidant constituents (Özen, 2010). The great reducing capacity of extract may be the result of its effective amount of phenols and flavonoid compounds. Like relations concerning Fe^{3+}-reducing ability and polyphenols have been declared in the literature (Gao et al., 2000).

Clearly, the ability of our plant to scavenge hydrogen peroxide could be due to the presence of flavonoid compounds and phenols. Similar studies suggested that the elimination of hydrogen peroxide is very necessary for the prevention of the living process (Koldaş et al., 2015).

The free radical scavenging activity was also increased with increasing extracts concentrations. Our data clearly indicated that the *n*-butanol extract of *P. coronopifolia* is a powerful free radical inhibitor. This result can consider to be noteworthy when compared to the finding of Boussaha et al. (2015) which suggests that this antioxidant ability can be attributed to extract's content of flavonoids like taxifolin, rhamnazin and caffeoylquinic acid derivatives determined in this plant.

The formation of ferrozine-Fe^{2+} complex is disrupted in the presence of *n*-butanol extract which indicates that it chelates iron. The metal chelating capacity of *n*-butanol extract was increased by increasing concentration. It was indicated that chelating compounds making α-bands with a metal are lesser anti-oxidants owing to reduction of the redox potential. It was reported in the literature that iron can provoke lipid peroxidation by Fenton-reaction, as can quicken it by decomposition of lipid hydroperoxides to peroxyl ($LO_2\cdot$) and alkoxyl ($LO\cdot$) radicals which may be able to capture hydrogen and propagate the formation of lipid peroxidation (Gutteridge, 1984).

ROS are known to have numerous damaging effects on the cells of the body. They severely induce lesions in DNA as deletions, mutations and genetic effects. Natural products in medicinal plants can scavenge the ROS generated by severing oxidative stress (Guha et al., 2011). Diverse study findings suggest that some plant extracts can protect DNA from oxidative damage by their antioxidant compounds (Arora et al., 2017). According to the reports, plant extract was practical to prevent DNA from oxidative injury induced by UV-photolysis of H_2O_2 (Lassed et al., 2015).

The current study was based on the ability of the *n*-butanol extract of *P. coronopifolia* to protect 46966 plasmid DNA against UV-photolysis of H_2O_2-induced oxidative damage. Exposure of plasmid to UV-photolysis of H_2O_2 results in generation of hydroxyl radicals (OH). These radicals are known to cause oxidatively breakage of DNA strands, fragmentation of deoxysugars, modification of bases, oxidation of lipids and generation of end products like malondialdehydes and other unsaturated aldehydes, which can bind to DNA and result in mutagenic adducts (Chaudhary et al., 1994). According to the cumulative level of DPPH· scavenging, antioxidant activity, reducing power, hydrogen peroxide scavenging and metal chelating assays, the extract may provide DNA protection.

The electrophoretic pattern of 46966 plasmid-DNA using UV-photolysis of H_2O_2 in the absence or presence of extract. At a dose of 50 μg the extract exhibited a complete protection of plasmid DNA, which indicates that the plasmid-DNA damaging is caused by hydroxyl radicals. The upon-treatment with *n*-butanol extract of *P. coronopifolia* protects the plasmid DNA from the hydroxyl radicals generated by UV-photolysis of H_2O_2. This investigation exhibits a noteworthy correlation between antioxidant potential and DNA protection. These results suggest that DNA loss inhibition may possibly owe to the great antioxidant capacity of the *n*-butanol extract of *P. coronopifolia.*

As compared with the positive control, the extract exposed varied profiles at different concentrations. HeLa cells were inactivated by *n*-butanol extract and showed the maximum activity at the concentration of 250 μg/mL. However, C6 cells recovered from the inhibition in few hours. This possibly due to the lower concentration of active biomolecules in the plant extract, then cells initiated to proliferate after a few hours of inhibition.

The activity of higher concentration might be due to the chemical profiles as the high conception of polyphenolic and flavonoid content (Yaglioglu et al., 2013). While the diminution of this capacity might cause by the low bioactive molecule content of the plant extract which degraded within the time, cells can continue to grow and reproduce after inactivation. This *n*-butanol extract-findings agreed with the results of another study which suggests that the ethyl acetate extract of the same plant has an antioxidant potent and can inhibit the proliferation of HeLa and C6-cell lines at high concentrations with no cytotoxic effect observed (Boussaha et al., 2015).

Conclusion

The *n*-butanol extract of *P. coronopifolia* has possessed a positive DNA-damage protection ability, offered by its significant anti-oxidant potential. As compared with 5-florourasil, the findings suggest that our extract has a positive anti-cancer activity against HeLa cells at high concentration.

Conflict of Interest

Authors declare no conflict of interest

References

Arora N, Tripathi D, Pandey N, Rai KK, Rai SK, Pandey-Rai S. Study of anti-oxidant, anti-inflammatory, and DNA-damage protection properties of some Indian medicinal plants reveal their possible role in combating psoriasis. Int J Appl Sci Biotechnol. 2017; 5: 141-49.

Barros L, Baptista P, Ferreira IC. Effect of *Lactarius piperatus* fruiting body maturity stage on anti-oxidant activity measured by several biochemical assays. Food Chem Toxicol. 2007; 45: 1731-37.

Boussaha S, Bekhouche K, Boudjerda A, Leon F, Koldas S, Yaglioglu AS, Demirtas I, Brouard I, Marchioni E, Zama D. Chemical constituents, *in vitro* anti-oxidant and anti-proliferative activities of *Perralderia coronopifolia* Coss. subsp. eu-coronopifolia M. var. typica M. extract. Rec Nat Prod. 2015; 9: 312-22.

Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I. Anti-oxidant principles from *Bauhinia tarapotensis*. J Nat Prod. 2001; 64: 892-95.

Casagrande R, Georgetti SR, Verri WA, Dorta DJ, dos Santos AC, Fonseca MJ. Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice. J Photochem Photobio B. 2006; 84: 21-27.

Chaudhary AK, Nokubo M, Reddy GR, Yeola SN, Morrow JD, Blair IA, Marnett LJ. Detection of endogenous malondialde-hyde-deoxyguanosine adducts in human liver. Science 1994; 265: 1580-82.

Collins AR, Gedik CM, Olmedilla B, Southon S, Bellizzi M. Oxidative DNA damage measured in human lymphocytes: Large differences between sexes and between countries, and correlations with heart disease mortality rates. FASEB J. 1998; 12: 1397-400.

Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D. Nicotera T. Oxidative damage to DNA in diabetes mellitus. Lancet 1996; 347: 444-45.

Demirtas I, Sahin A, Ayhan B, Tekin S, Telci I. Antiproliferative effects of the methanolic extracts of *Sideritis libanotica* Labill. subsp *linearis*. Rec Nat Prod. 2009; 3: 104-09.

Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 1994; 315: 161-69.

Gao X, Björk L, Trajkovski V, Uggla M. Evaluation of anti-oxidant activities of rosehip ethanol extracts in different test systems. J Sci Food Agric. 2000; 80: 2021-27.

Guha G, Rajkumar V, Ashok Kumar R, Mathew L. Therapeutic potential of polar and non-polar extracts of *Cyanthillium cinereum in vitro*. Evid Based Complement Alternat Med. 2011; 2011: 1-10.

Gutteridge JM. Ferrous ion-EDTA-stimulated phospholipid peroxidation. A reaction changing from alkoxyl-radical-to hydroxyl-radical-dependent initiation. Biochem J. 1984; 224: 697-701.

Koldaş S, Demirtas I, Ozen T, Demirci M A, Behçet L. Phyto-chemical screening, anti-cancer and anti-oxidant activities of *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek, a plant of traditional usage. J Sci Food Agric. 2015; 95: 786-98.

Kumar A, Pant MC, Singh HS, Khandelwal S. Assessment of the redox profile and oxidative DNA damage (8-OHdG) in squamous cell carcinoma of head and neck. J Canc Res Ther. 2012; 8: 254-59.

Kumar PS, Sucheta S, Deepa VS, Selvamani P, Latha S. Anti-oxidant activity in some selected Indian medicinal plants. Afr J Biotechnol. 2008; 7: 1826-28.

Lassed S, Amrani A, Altun M, Zama D, Demirtas I, Benayache F, Benayache S. *In vitro* anti-oxidant, inhibition of oxidative DNA damage and antiproliferative activities of ethanolic green tea (*Camellia sinensis*) extract. Int J Pharm Sci Rev Res. 2015; 35: 36-42.

Lezza A, Mecocci P, Cormio A, Beal MF, Cherubini A, Cantatore P, Senin U, Gadaleta M. Area-specific differences in OH8dG and mtDNA4977 levels in Alzheimer disease patients and aged controls. J Anti-Aging Med. 1999; 2: 209-16.

Oyaizu M. Studies on products of browning reaction. Jpn J Nutr. 1986; 44: 307-15.

Ozen T, Darcan C, Aktop O, Turkekul I. Screening of anti-oxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea region of Turkey. Comb Chem High T Scr. 2011; 14: 72-84.

Özen T. Anti-oxidant activity of wild edible plants in the Black Sea Region of Turkey. Grasas Aceites. 2010; 61: 86-94.

Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of anti-oxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999; 269: 337-41.

Russo A, Izzo A, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomedicine 2001; 8: 125-32.

Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and anti-oxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999; 299: 152-78.

Souri E, Amin G, Farsam H. Screening of anti-oxidant activity and phenolic content of 24 medicinal plant extracts. DARU J Pharml Sci. 2008; 16: 83-87.

Wang H, Gao XD, Zhou GC, Cai L, Yao WB. *In vitro* and *in vivo* anti-oxidant activity of aqueous extract from *Choerospondias axillaris* fruit. Food Chem. 2008; 106: 888-95.

Yaglıoglu AS, Akdulum B, Erenler R, Demirtas I, Telci I. Tekin S. Anti-proliferative activity of pentadeca-(8E, 13Z) dien-11-yn-2-one and (E)-1, 8-pentadecadiene from *Echinacea pallida* (Nutt.) Nutt. roots. Med Chem Res. 2013; 22: 2946-53.

Zhao GR, Xiang ZJ, Ye TX, Yuan YJ, Guo ZX. Anti-oxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem. 2006; 99: 767-74.

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