


Bongardia chrysogonum (L.) Spach as a potential medicinal plant against cancer and Alzheimer's disease management

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ABSTRACT

Background and Aims: *Bongardia chrysogonum* (L.) Spach (Berberidaceae), an ancient medicinal plant in Eastern Mediterranean countries, is traditionally used for the treatment of epilepsy, hemorrhoids, urinary system infections and gastrointestinal disorders. This study was designed to evaluate potential neuroprotective and anticancer effects of different parts from *B. chrysogonum*.

Material and Methods: Leaf, stem, and tuber parts of the plant were extracted and subjected to determine cholinesterase enzyme inhibitory potentials and anticancer activities. Enzyme inhibition assays were carried out to screen neuroprotective potentials, while the MTT assay was performed for evaluating anticancer activities of the extracts towards human lung carcinoma (A549), breast adenocarcinoma (MCF-7), brain glioma (C6), and non-tumorous human umbilical vein endothelial cell (HUVEC) lines.

Results: Among the tested extracts, the highest enzyme inhibitory activity was exhibited by tuber-water extract (83.81±0.33% and 62.14±0.60% inhibition on AChE and BChE at 400 µg/mL p<0.01, respectively), whilst the lowest enzyme inhibition was exerted by the tuber-chloroform extract. Moreover, the tuber part of the plant was found to have the most cytotoxic activity against all the cancer cells, and the best anticancer activity was determined in tuber ethanol extract against MCF-7 cells (IC₅₀= 28.36±0.04, p<0.01).

Conclusion: To the best of our knowledge, this research is the first study that assessed the *in vitro* neuroprotective effect of the aerial parts and tuber extracts of the plant through inhibition of cholinesterase enzymes alongside an anticancer capacity towards human cancer cells. The results revealed that this plant is a good candidate for performing further clinical studies in cancer and neurodegenerative diseases.

Keywords: *Bongardia chrysogonum* (L.) Spach, cancer, enzyme inhibition, medicinal plant, neuroprotection

INTRODUCTION

Medicinal plants used in traditional medicine are well-known to have numerous pharmacological actions and extraordinary therapeutic potentials in the prevention and cure of several diseases, particularly oxidative stress related-diseases including diabetes, hypertension, atherosclerosis, cancer, ischemia, multiple sclerosis, cardiac hypertrophy, amyotrophic lateral sclerosis, Alzheimer's, and Parkinson's diseases (Fang *et al.*, 2017; Ismail, Amanat, Iqbal & Mirza, 2018; Gezici & Sekeroglu 2019a; Ahmad, Waheed, Rozeen, Mahmood & Kamal, 2019. Moreover, medicinal plant-derived natural compounds serve as a therapeutic alter-

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native for combating cancer and neurodegenerative disorders, owing to rich phytochemical contents and natural antioxidant potentials (Safarzadeh, Shotorbani & Baradaran, 2014; Chen, Lin, Dong, Zhang & Du, 2018; Sahoo, Dandapat, Dash & Kanhar, 2018; Gezici & Sekeroglu, 2019b). According to statistical data, there has been a great increase in Alzheimer's disease (AD) incidence around the world, from this point of view, finding effective and safe therapy approaches have an accountable role to manage the high incidence of this disease. On the other hand, cholinergic-based therapy that means inhibition of the level of some enzymes including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) seems to be as one of the most effective treatment strategies for the AD pathogenesis (Ismail *et al.*, 2018; Harris, 2019; Gezici, 2018). In this concern, medicinal plants and natural products have been gaining interest among the researchers almost all over the world. Thus, the researchers have focused on the development of novel plant-derived natural products with less or no side effects as well as unlimited availability.

The genus *Bongardia* L. (Berberidaceae), native to the Eastern Mediterranean region, is represented by only one species (*Bongardia chrysogonum*) in the flora of Turkey. This species is generally known as 'Uruf-el-Deek' and locally known as 'catlak otu' in Anatolia, which is distributed in the Eastern Mediterranean region, North America, Jordan, Afghanistan, Syria, Iran, Iraq, Azerbaijan, and Turkey. Tubers of this plant have been mainly used in folk remedy since ancient times (Karl & Strid, 2009; Abuhamdah *et al.*, 2017a; Abuhamdah *et al.*, 2017b). From the past to today, scientific records have stated that the tubers of *B. chrysogonum* have been utilized for the treatment of epilepsy and hematological malignancies in the form of decoction (2-3%) around Afghanistan, Iran, Jordan, Syria, and Turkey by the local rural communities. The tubers have also been reported to be used in order to treat gastrointestinal disorders, urinary tract infections, prostate hypertrophy, hypercholesterolemia, hemorrhoids, and diabetes, because of their antidiarrheal, antimicrobial, antiulcerogenic, anticonvulsant, and antioxidative activities (Alfatafta, Abu Zarga, Sabri, Freyer & Shamma, 1989; Baydoun, Chalak, Dalleh & Arnold, 2015; Dokuyucu *et al.*, 2016; Abuhamdah *et al.*, 2017a; Abuhamdah *et al.*, 2017b).

Recent studies reported the antioxidant and anti-convulsant activities of the tuber extracts of *B. chrysogonum* (Abuhamdah *et al.*, 2017a; Abuhamdah *et al.*, 2017b). However, the other biological activities have not been identified using each part of this plant, yet. In this context, extracts of the aerial parts (leaf and stem, individually) and tuber of *B. chrysogonum* extracted by using different solvents including ethanol, water, and chloroform were screened for revealing potential anticancer and neuroprotective capacities in the presented work. The neuroprotective potential of the extracts was evaluated through AChE and BChE inhibitory activities. The MTT assay was performed for determining cytotoxic and anticancer activities of the extracts against cells; A549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), C6 (human brain glioma), and non-tumorous HUVEC (human umbilical vein endothelial cells), in a concentration and time dependently.

MATERIALS AND METHODS

Chemicals and equipment

5,5-dithio-bis-2-nitrobenzoic acid (DTNB), ethanol (EtOH), methanol (MeOH), chloroform (CHCl₃), dimethyl sulfoxide (DMSO), neocuproine, ammonium acetate (NH₄Ac), AChE (electric eel acetylcholinesterase, Type-VI-S, EC 3.1.1.7), BChE (horse serum butyrylcholinesterase, EC 3.1.1.8), acetylthiocholine iodide (ATCI), butyrylthiocholine chloride (BTCl), sodium dihydrogen phosphate (NaH₂PO₄), sodium bicarbonate (NaHCO₃), and reference standards were purchased from Sigma (St. Louis, MO, USA). Cell culture mediums and chemicals were purchased from ThermoFisher Scientific, Gibco®. A Microplate reader spectrophotometer from Thermo Lab systems (408 Multiskan) was used to measure the absorbance.

Collection of plant material

The plant sample used in this study was collected from its natural habitat from the Gaziantep province (Turkey) in April, 2018. The plant species was identified by Res. Assist. Fatih Yayla, a taxonomist from the Department of Biology, Gaziantep University, Gaziantep (Turkey). A voucher specimen (number: GAUN1603) was deposited at the Herbarium of Department of Biology, Gaziantep University, Turkey (Figure 1).



Figure 1. *Bongardia chrysogonum* a) aerial parts, b) tuber.

Preparation of the extracts

The air-dried plant material including the tuber and aerial parts (leaf and stem) of *B. chrysogonum* was extracted with 70% ethanol (EtOH), distilled water (dH₂O), and chloroform (CHCl₃) for two days at room temperature as described in the previous publications (Gezici & Sekeroglu, 2019a; Gezici, 2019). After the extraction procedures, the extracts were deposited at -20°C until further analysis.

AChE and BChE enzyme inhibitory studies

The Neuroprotective potential of the extracts expressed as their inhibitory activity against cholinesterase enzymes (AChE and BChE) was determined by as described by Ellman, Courtney, Andres & Featherstone, (1961) and all conditions were similar to that described previously (Senol, Sekeroglu, Gezici, Kilic & Orhan, 2018; Sekeroglu & Gezici, 2019). The reaction was observed at 412 nm utilizing a microplate reader spectrophotometer, using galanthamine as the reference drug.

The percent inhibition of the enzymes was calculated by a comparison of reaction rates in the extract with a blank sample using the formula as given below:

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

[Where A_{enzyme} is the activity of enzyme without the extract, and A_{sample} is the activity of enzyme with the extract].

Anticancer activity studies

Cell lines and cultures

A549 (lung carcinoma), MCF-7 (breast adenocarcinoma), C6 (brain glioma), and non-tumorous HUVEC (human umbilical vein endothelial cells) cell lines, obtained from the American Type Culture Collection (ATCC, USA) were used to determine the *in vitro* anticancer effects of *B. chrysogonum* extracts. The lung carcinoma cell was cultured on Roswell Park Memorial Institute Medium (RPMI), supplemented with 10% (v/v) fetal bovine serum (FBS), antibiotics (1%, 100 U/ml penicillin and 100 µg/ml streptomycin), and L-glutamine (1%) in the flasks at 37°C in a humidified CO₂ (5%) incubator. MCF-7, C6, and HUVEC cells were grown in Dulbecco's modified Eagle medium (DMEM): Ham's F12 nutrient medium (1:1) with the same additives. All the assays were carried out in triplicate using the cell lines from passage 24 or less.

MTT anticancer activity assay

To evaluate the cytotoxic potentials of the extracts, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed as described by Mosmann (1983) with a minor modification (Gezici, Sekeroglu & Kijjoa, 2017; Gezici, 2019). Briefly, densities of 5×10^3 cells were seeded in 200 µl medium into 96-well plates for 24 hrs and after seeding, the extracts with concentrations from 50 µg/mL to 400 µg/mL were added into all the wells, and then they incubated at 37°C for 48 hrs. After incubation, the medium was discarded and 50 µL/well of MTT (Sigma-Aldrich) solution (5 mg/mL) was added into each well and incubated for 4 hrs at 37°C. The medium-containing MTT was discarded and 200 µL of DMSO added for both lysis the cells and solubilization formazone. Then, the absorbance was measured at 570 nm with a Thermo Lab system 408 Multiskan multiplate spectrophotometer. The dose-response curve was used to generate the IC₅₀ (µg/mL) values, and doxorubicin used as the reference.

Statistical analysis

The experiments were carried out in triplicate and data were presented as mean±S.D. values (n=3). A linear regression analysis was conducted for generating IC₅₀ values. ANOVA (one way) was used for the evaluation of statistical differences between the control and the sample groups. A *p* value of <0.05 was examined to be significant, and *p* <0.01 was considered to be very significant statistically (**p* < 0.05, ***p* < 0.01).

RESULTS

AChE and BChE enzyme inhibitory results

All the extracts from the leaf, stem, and tuber of *B. chrysogonum* were evaluated for their inhibitory activities against AChE and BChE enzymes at 50, 100, 200, and 400 µg/mL concentrations, and galanthamine was used as the reference drug (Table 1). As

Table 1. Inhibitory activity of the extracts against AChE and BChE enzymes.

Plant parts	Extract type	AChE				BChE			
		50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL ^b	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL ^b
Leaf	Ethanol	45.01±0.56*	52.28±1.15**	60.14±0.52**	68.93±0.22**	14.08±0.66*	19.52±0.84**	27.97±1.01*	33.06±1.02**
	Water	36.32±0.48**	41.27±0.62*	51.96±0.71**	62.10±0.45**	12.35±0.92*	20.91±0.52*	26.02±0.12**	31.48±0.83*
	Chloroform	---	---	---	---	---	---	---	---
Stem	Ethanol	47.85±1.10*	59.16±0.91**	65.08±0.36**	72.16±1.02**	22.02±0.14**	34.77±0.60*	41.68±0.63**	49.85±0.15**
	Water	38.20±0.21**	44.36±0.13*	49.71±1.19*	65.04±1.16**	17.54±0.67**	23.45±0.42**	30.06±0.82*	36.11±0.78*
	Chloroform	---	---	---	---	---	---	---	---
Tuber	Ethanol	50.25±1.05**	63.04±1.20**	70.16±0.65*	81.45±0.42**	27.61±1.01**	35.69±0.76*	44.12±0.16**	51.20±1.17**
	Water	54.21±1.23**	66.05±1.42*	74.92±0.82**	83.81±0.33**	32.98±0.78**	43.01±1.20**	50.16±0.47*	62.14±0.60**
	Chloroform	23.45±0.14**	27.61±0.65**	31.04±0.93**	43.86±0.90*	9.96±0.08**	17.26±0.18**	23.18±0.59**	27.63±1.09*
Galanthamine ^d		82.64±1.08* at 100 µg/mL			86.04±0.45* at 100 µg/mL				

*S.D.: Standard deviation (n=3). ^aThe final concentration in the well. ^bNo inhibition. ^cGalanthamine; reference for AChE and BChE enzymes. **p* value of < 0.05; ***p* value of < 0.01

summarized in Table 1, the extracts tested herein demonstrated from moderate to a high level of inhibition against the above-mentioned enzymes. The extracts inhibited AChE at high levels, whilst they inhibited BChE at moderate levels (below 50%). Although a significant variation was not assessed among the tested extracts, the tuber water extract showed a significant inhibition against AChE and BChE ($83.81 \pm 0.33\%$ and $62.14 \pm 0.60\%$, respectively) at $400 \mu\text{g/mL}$. The tuber chloroform extract was exerted the lowest enzyme inhibition through AChE and BChE ($43.86 \pm 0.90\%$ and $27.63 \pm 1.09\%$, respectively), and the chloroform extracts from leaves and stems were also observed to be ineffective in inhibiting both cholinesterase. In the assay, the water extracts from the leaves and stems exhibited moderate inhibitory activities on both AChE and BChE enzymes in the range of $31.48 \pm 0.83\%$ and $65.04 \pm 1.16\%$ as compared to those of the ethanol extracts (Table 1).

Anticancer activity results against human cancer cells

The cytotoxic effects of the mentioned plant parts of *B. chrysonum* were assessed against A-549, MCF-7, and C6 cancer cells, in comparison to non-tumour HUVECs. The tested extracts exerted a noteworthy cancer prevention potential against the cancer cells in a dose and time dependent manner; however, the IC_{50} values were varied depending on the cancer cells and plant parts. The results were expressed as IC_{50} ($\mu\text{g/mL}$) values after 72 hrs treatment period at $200 \mu\text{g/mL}$ concentration (Table 2).

As concluded from the IC_{50} values given in Table 2, the tuber part of the plant was found to possess higher cancer prevention potentials, when compared to those of the aerial parts. In all cases, all plant parts caused a much more cytotoxicity on MCF-7 cells, while they exhibited lower effects against C6 cells. On the other hand, the tuber EtOH extract showed the best

Table 2. Anticancer activities of the extracts against A549, MCF-7, and C6 human cancer cells.

Human cancer cells	Plant parts	Extract type	IC_{50} values ^a ($\mu\text{g/mL}$)
A549	Leaf	Ethanol	$190.66 \pm 1.47^*$
		Water	$145.02 \pm 0.96^{**}$
		Chloroform	$228.16 \pm 1.03^*$
	Stem	Ethanol	$235.16 \pm 1.92^{**}$
		Water	$201.80 \pm 2.07^*$
		Chloroform	$280.94 \pm 0.39^*$
	Tuber	Ethanol	$147.01 \pm 0.82^{**}$
		Water	$80.06 \pm 1.25^{**}$
		Chloroform	$178.12 \pm 1.94^{**}$
MCF-7	Leaf	Ethanol	$74.48 \pm 1.01^*$
		Water	$98.07 \pm 0.19^*$
		Chloroform	$129.35 \pm 0.43^{**}$
	Stem	Ethanol	$174.27 \pm 0.92^{**}$
		Water	$191.95 \pm 2.06^{**}$
		Chloroform	$237.03 \pm 2.54^*$
	Tuber	Ethanol	$28.36 \pm 0.04^{**}$
		Water	$61.23 \pm 1.10^{**}$
		Chloroform	$69.72 \pm 0.38^{**}$
C6	Leaf	Ethanol	$285.24 \pm 0.68^{**}$
		Water	$259.09 \pm 2.06^*$
		Chloroform	$306.75 \pm 1.94^{**}$
	Stem	Ethanol	$286.10 \pm 0.71^*$
		Water	$262.55 \pm 1.32^{**}$
		Chloroform	$321.68 \pm 2.14^*$
	Tuber	Ethanol	$240.92 \pm 1.19^{**}$
		Water	$214.50 \pm 0.25^{**}$
		Chloroform	$287.01 \pm 0.52^{**}$
Doxorubicin ^b		7.96 ± 0.18	
DMSO (dimethyl sulfoxide) ^c		0	

^aValues were expressed as $\text{IC}_{50} \pm \text{S.D.}$ from three independent experiment ($n=3$). ^bDoxorubicin, positive control. ^cDMSO; negative control. * p value of < 0.05 ; ** p value of < 0.01 .

anticancer activity ($IC_{50} = 28.36 \pm 0.04 \mu\text{g/mL}$, $p < 0.01$) towards MCF-7 human cancer cells, when the stem chloroform extract was found to have the weakest anticancer activity against C6 cells ($IC_{50} = 321.68 \pm 2.14 \mu\text{g/mL}$, $p < 0.05$). As for the extraction type, the water extracts obtained from any part of the plant demonstrated the highest anticancer activity towards A549 and C6 cancer cells. However, the EtOH extracts owned the highest cancer prevention capacities against MCF-7 cells, whereas the chloroform extracts showed the lowest ones in all cases.

DISCUSSION

Acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase (TYR) enzymes involved in neurodegenerative mechanisms have been reported to be associated with pathogenesis of neurodegeneration and neurodevelopmental diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), prion diseases, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (ASD). Of which, AD is known as the most common type of neurodegenerative disorder leading to a loss of memory and behavior skills in the elderly (Alamgir, 2017; Ismail *et al.*, 2018; Ahmad *et al.*, 2019). A number of medicinal plants and plant-derived compounds have been reported to inhibit AChE and BChE, involved in the acetylcholine and butyrylcholine hydrolysis, respectively, which is the most accepted drug treatment strategy in patients with AD (Grutzendler & Morris, 2001; Sekeroglu *et al.*, 2012; Gezici & Sekeroglu, 2019a). From this point, the inhibition of key enzymes linked to the pathogenesis of the AD and PD diseases used medicinal plant and plant-derived compounds are suggested as one of the most effective strategies. For example, the inhibition of AChE and BChE, involved in the acetylcholine and butyrylcholine hydrolysis, respectively is the most accepted treatment strategy in AD. Additionally, the inhibition of TYR alongside cholinesterase (ChE) is of great importance for the treatment of these diseases as multi-target drug approaches (Sekeroglu *et al.*, 2012; Santos, Gomes, Pinto, Camara & Paes, 2018; Gezici & Sekeroglu, 2019a; Gezici & Sekeroglu, 2021).

In our ongoing research on finding natural enzyme inhibitors, neuroprotective potentials of *B. chrysogonum* extracts through AChE and BChE enzyme inhibition were aimed to screen in this work. Based on the results of cholinesterase enzyme inhibition, the tested extracts of the plant were found to exhibit a promising enzyme inhibitory effect towards both AChE and BChE, which are closely associated with AD development. Probably, these strong enzyme inhibition potentials of this plant may be due to the presence of rich secondary metabolites including bongardine, N-acetyl bongardine, bongardol, bongardol acetate, and bongardamine, as reported by previous studies (Alfatafta *et al.*, 1989; Rahman *et al.*, 1998; Shahwar, Choudhary, Sener, Toker & Baser, 1999; Rahman *et al.*, 2000; Karl & Strid, 2009). In addition, alkaloids are important secondary metabolites for cholinesterase inhibition. Previous studies showed that this plant is also rich for isoquinoline alkaloids like retuculine, coclaurine etc. (Al-

fatafta *et al.*, 1989; Abuhamdah *et al.*, 2017a; Abuhamdah *et al.*, 2017b).

The increasing cancer burden worldwide requires an alternative treatment solution. Plants have been used for medicinal purposes since times immemorial, and these plants and natural compounds isolated or extracted from the medicinally important plants offer a very viable alternative in the treatment and management of several cancer types (Roleira *et al.*, 2015; Gezici & Sekeroglu, 2019b; Khan *et al.*, 2020). Based on their traditional uses and experimental evidences, cancer prevention potentials of *B. chrysogonum* were analyzed in the presented research. Particularly, the tuber parts of *B. chrysogonum* extracts prepared using different solvents demonstrated a good level of anticancer effects against the tested cancer cells. According to the anticancer activity results, increasing concentration of the extracts resulted in a decrease in the number of cancer cells, likewise, an increasing exposure time resulted in a significant inhibition of cell growth in the cancer cells. It is clearly proven that the plant could have a noteworthy potential as an anticancer agent for the management of reducing the number of cancer cells and suppressing the cell growth in cancer. In the light of the literature, the notable cancer prevention effects of *B. chrysogonum* are most likely due to the fact that it has rich phytochemical contents such as isoquinoline alkaloids and triterpenoid saponins as revealed previously (Alfatafta *et al.*, 1989; Rahman *et al.*, 1998; Shahwar *et al.*, 1999; Rahman *et al.*, 2000; Karl & Strid, 2009).

Although, 2,2- diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl ($\cdot\text{OH}$) free radical scavenging activities of the tuber part of *B. chrysogonum* have been demonstrated by a few previous studies until now (Dokuyucu *et al.*, 2016; Abuhamdah *et al.*, 2017a; Abuhamdah *et al.*, 2017b), there has been no research conducted for the evaluation of anti-cholinesterase and anticancer effects using individually each part of *B. chrysogonum* in the literature. Therefore, to the best of our knowledge, the assessment of *in vitro* neuroprotective and cancer preventive potentials of different plant parts of *B. chrysogonum* were performed for the first time in the current research.

CONCLUSION

To summarize, the probable *in vitro* neuroprotective capacities and anticancer effects of the ethanol, water, and chloroform extracts from different parts of *B. chrysogonum* (L.) Spach were evaluated the first time in the current study. The results obtained exhibited that the tuber part of the plant has the greatest biomedical potential through its significant inhibitory activities on the tested enzymes related to AD and also a remarkable cancer prevention activity against A549, MCF-7, and C6 cancer cells. These findings could be of valuable scientific evidence to contribute to the pharmaceutical industry in the prevention and treatment of AD and cancer; however, detailed mechanisms based *in vitro* and *in vivo* studies are necessary in future. Besides, in order to specify the phytochemicals responsible for notable neuroprotective effect and cancer prevention capacity of the plant-extracts, phytochemistry of the extracts should be analyzed as further research.

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

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