Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Determination of inhibitory activities of enzymes, related to Alzheimer's disease and diabetes mellitus of plane tree (*Platanus orientalis* L.) extracts and their antioxidant, antimicrobial and anticancer activities

Esra Ucar[1*], Nuraniye Eruygur[2], Mehmet Atas[3], Merve Ergul[4], Mustafa Ergul[5], Fazli Sozmen[6]

[1] Medicinal and Aromatic Plant Department, Vocational School, Cumhuriyet University, 58140, Sivas, Turkey
[2] Pharmacognosy Department, Faculty of Pharmacy, Selcuk University, 42250, Konya, Turkey
[3] Pharmaceutical Microbiology Department, Faculty of Pharmacy, Cumhuriyet University, 58140, Sivas, Turkey
[4] Pharmacology Department, Faculty of Pharmacy, Cumhuriyet University, 58140, Sivas, Turkey
[5] Biochemistry Department, Faculty of Pharmacy, Cumhuriyet University, 58140, Sivas, Turkey
[6] Nanotechnology Engineering Department, Faculty of Engineering, Cumhuriyet University, 58140, Sivas, Turkey

Correspondence to: eucar@cumhuriyet.edu.tr

Received February 26, 2018; **Accepted** August 2, 2018; **Published** August 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.11.3

Abstract: Plane tree (*Platanus orientalis* L.) leaves have been employed for centuries in various countries due to their pharmacological value. Therefore, determination of the biological activity of the leaves is of interest. The aim of the study was to evaluate the inhibitory effects against Alzheimer's disease-related enzymes Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE), diabetes mellitus related enzymes α-glucosidase and α-amylase. The antioxidant, anticancer, and antimicrobial activities of the leaves were also studied. According to the results, both water and methanol extracts of *P. orientalis* demonstrated more α-glucosidase and α-amylase inhibition activity than the antidiabetic drug-acarbose at the same concentration level. In addition, extracts showed good inhibition activity against AChE and BuChE. Significant results were obtained regarding antioxidant, anticancer, and antimicrobial activities. These results are very promising especially for the improvement of pharmaceutical formulations to treat various diseases such as age-related diseases, cancer, diabetes etc. and it is necessary to conduct further experiments.

Key words: Plane tree; Alzheimer's Disease; Diabetes; Antioxidant Activity; Cytotoxic and Antimicrobial activities.

Introduction

The *Platanus orientalis* L. (plane tree) is a member of the Platanaceae family and grows naturally in the Mediterranean region of Southern-Europe and south-western Asia especially in microclimate regions. The *P. orientalis* has deeply lobed leaves and very strong branches (1, 2). The leaves of *P. orientalis* possess several active compounds, such as flavonoids, pentacyclic triterpenoids, tannins, and caffeic acid (3-6). These compounds are widely used as traditional medicine in some countries such as Turkey and Iran for the treatment of some dermatological, gastrointestinal, rheumatic, and inflammatory diseases (3, 7).

Today's plant medicines and natural products, compared to chemical drugs, are great importance and more attention to them (8). Today, it is well known that many diseases such as cancer, autoimmune disorders, ageing, cardiovascular, and neurodegenerative diseases are directly related to oxidative stress and antioxidants are the compounds that can prevent or stop cell damage caused by oxidants. In other words, they improve the body's cellular defence system against oxidative damage. Therefore, they are extremely important for our health and they are readily available from natural sources, especially plants.

Cholinesterases, which are a family of enzymes, catalyse the hydrolysis of acetylcholine (ACh) into choline and acetic acid (9). There are two main types of cholinesterase, one of which is acetylcholinesterase (AChE; EC 3.1.1.7.) and the other is butyrylcholinesterase (BChE; EC 3.1.1.8). Although AChE and BChE are similar to each other, in the body their importance and localization show differences (9). Studies have demonstrated that AChE plays a significant role in the acceleration of amyloid deposition in the brain in Alzheimer's disease (10). Therefore, cholinesterase inhibitors (ChEI) are used to stabilize the cognitive functions of Alzheimer's disease patients and ChEI can prolong the stabilization up to 24 months in some patients (10).

α-glucosidase and α-amylase, which are the main starch digestive enzymes, directly affect the glucose level in blood. Thus, to decrease the postprandial increase of blood glucose, their inhibition is a sensible strategy, especially for the treatment of type 2 diabetic patients (11, 12). Some synthetic drugs used for inhibition of these enzymes may cause some gastrointestinal side-effects, so as with many new drug discoveriesy, it is crucial to obtain drugs from natural products.

Cancer is a very serious life-threatening disease and the second-leading cause of death worldwide after cardiovascular diseases. Traditional cancer treatments including chemotherapy, surgery, and radiotherapy have several side-effects and may cause systemic toxicity.

In addition, the resistance of cancer cells to chemotherapeutic drugs is an important problem (13). Consequently, the usage of natural products has significantly increased in recent years (14).

The aim of this study was to identify and compare the chemical content of extracts obtained from water and methanol extraction of *P. orientalis* leaves. To be able to understand the pharmacological value of *P. orientalis* leaves, the antimicrobial, anticancer, antioxidant, and various enzyme inhibition activities of these two extracts were also studied. To the best of our knowledge, this is the first report to have demonstrated the antimicrobial, anticancer, antioxidant, and various enzyme activities of extracts of *P. orientalis* leaves.

Materials and Methods

This study was conducted in the laboratories of the Faculty of Pharmacy, Cumhuriyet University, Sivas in 2018. Leaves of *P. orientalis* L. were collected from the wild flora of Sivas. The experiments were performed in completely randomized design with three replications.

MDA-MB-231 cells, derived from human breast adenocarcinoma, and L929, derived from mouse fibroblast cells, were obtained from the American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM) (ATCC, Manassa, USA), fetal bovine serum (FBS) and sterile phosphate buffer saline (PBS) were obtained from PAA Ltd. (France). Trypsin-EDTA was purchased from Biological Industries Ltd. (Haemek, Israel). DMEM without phenol red and L-glutamine–penicillin streptomycin solution were purchased from Sigma-Aldrich (Steinheim am Albuch, Germany). XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was purchased from Roche Diagnostic.

Preparation of extracts

The plant leaves were milled with a grinder then dried to a constant weight out of sunlight. The dry plant leaves were ground in a blender (Blue house). 10 gr of the leaf was soaked in 50 mL of methanol (Sigma) for 24 h with intermittent shaking. At the end of the extraction, it was filtered through No. 1 Whatman filter paper. The filtrate was concentrated to dryness under reduced pressure in a rotary evaporator at 40°C and this was repeated three times. The obtained extracts were analyzed using Gas Chromatography Mass Spectrometry (15).

In-vitro Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of the extracts were determined using the Clarke, G. method (16). 20 µL of test solutions diluted with DMSO was mixed with 180 µL of DPPH solution (in 40 µg/mL concentration prepared in methanol) in 96-well plates. After the plates were left in the dark for 15 min, the absorbance was read with an ELISA reader at 540 nm. Quercetin solution prepared with DMSO instead of the test sample as standard and DMSO as control were run in parallel. The experiments were conducted in parallel in three groups and the standard average error (SEM) was also calculated. The results were expressed as % DPPH sweeping effect using the following equation:

% DPPH Scavenging Effect = ((Control Absorbance - Sample Absorbance)/Control Absorbance)) x 100

Where control absorbance was without test substances, the sample absorbance was the extract/quercetin absorbance.

2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay

The method of Chun et al. was used to determine the ABTS scavenging activity of the plant extracts (17). Test samples were prepared as in the DPPH method. The stock solution of ABTS + radical was prepared by allowing 15 mL of 7 mM ABTS and 264 µL of 140 mM potassium persulfate solution to stand in the dark at room temperature for 16 h before experimentation. The ABTS working solution was adjusted so that the previously prepared stock solution had an absorbance of 0.70 ± 0.02 at 734 nm with methanol dilution. On a 96-well plate, 50 µL sample solution was mixed with 100 µL ABTS working solution. After the mixture was allowed to stand at room temperature for 10 min, the absorbance was read at 734 nm. The ABTS + scavenging activity of the plant extract was compared with BHT and the percentage inhibition was calculated with the formula:

$ABTS^{+}$ scavenging activity (%)=[($Abs_{control}$-Abs_{test})/($Abs_{control}$)] X 100

$Abs_{control}$: Absorption of ABTS + radical solution and methanol

Abs_{test}: Absorption of ABTS + radical and test or reference

Total phenol content

The total amount of phenol in the extracts was measured as the result of the reaction with the Folin-Ciocalteu (F-C) reagent. The extracts, diluted appropriately with 10 µL DMSO, was mixed with 100 µL of distilled water and freshly diluted 10-fold diluted F-C reagent. After 5 min, 100 µL of 7.5% Na_2CO_3 was added, then after a 60 min wait, the absorbance at 650 nm was measured. Appropriate blank (DMSO) and reference (Gallic acid in DMSO) were run in parallel. Total phenol quantities were then calculated from the absorbance values of the samples (16).

Total flavonoid content

The aluminum chloride colourimetric method was used to determine total flavonoid content in the extracts. For calibration, serial dilution solutions of 0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1 mg/mL were prepared from 10 mg/mL stock solution of quercetin. The test solution (150 µL, 0.3 mg/mL) prepared with ethanol was mixed on a 96-well plate with 2% $AlCl_3$. After 15 min incubation at room temperature, the absorbance was read at 435 nm. Quantities of total flavonoids were calculated as mg equivalent of quercetin over the dry weight of extract (18).

Iron chelating activity

The iron chelating activity of the extracts was determined according to their interaction with the formation of ferrozine-Fe^{2+} complex. Previously described procedures were used (19). Ferrozine, used in this method,

is a substance that can rapidly react with iron to form a coloured complex. This assay was performed by adding 200 µL of $FeCI_2$ (0.1 mM), 200 µL of extract, and 400 µL of 0.2 mM ferrozine in test tubes and allowing the mixtured to react at 25 ºC. After 10 min at room temperature, the absorbance of the assay samples was measured at 562 nm spectrophotometrically. EDTA was used as the positive control.

Ferric Reducing Antioxidant Power (FRAP) assay

FRAP assay uses antioxidants as reductants in a redox-linked colourimetric method. The FRAP assay was conducted according to the previously reported method with a slight modification (18, 20). Each extract was dissolved in DMSO to prepare a stock solution. FRAP reagents included 300 mmol/ litre acetate buffer, pH 3.6, 10 mmol/litre TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/litre HCI, and 20 mM $FeCI_3.6H_2O$ solution. The working FRAP reagent was prepared as required by mixing the solutions in the ratio of 10:1:1 at the time of use. All solutions were prepared fresh on the day of the experiment. 30 µL of the sample solution and 270 µL of freshly prepared FRAP reagent were mixed together in 96-well plates and warmed at 37ºC for 4 min. All determinations were performed in triplicate. The absorbance was measured at 593 nm. A standard calibration curve was prepared using different concentrations of $FeSO_4$ solution. The results were expressed as FRAP value.

Acetylcholinesterase / butyrylcholinesterase inhibition assay

The assay was carried out according to the Ellman method (21) as follows. The mixture consisting of 20 µL of test sample/reference standard of various concentrations, 140 µL of 200 mM phosphate buffer (pH 7.7), 10 µL of DTNB and 20 µL of enzyme (0.22 U/mL for acetylcholinesterase/ 0.1 U/mL for butyrylcholinesterase) was incubated for 5 min at 25 °C. Following preincubation, 10 µL of the substrate (0.71 mM acetylthiocholine iodide/0.2 mM butyrylthiocholine chloride in phosphate buffer) was added to start the reaction and incubated again for 10 min. The developed yellow colour was measured at 412 nm (Epoch, USA). Galanthamine was used as the positive control.

α-glucosidase inhibition activity

The α-glucosidase inhibition method was performed as previously described in literature (22). Acarbose was used as a positive control, while phosphate buffer was used as a negative control in place of the sample. Each concentration was carried out in triplicate. 25 µL of sample solution diluted with buffer was mixed with 25 µL of a-glucosidase (0.5 U/mL), and incubated for approximately 10 min at 25ºC. Then 25 µL of 0.5 mM 4-nitrophenyl-β-D-glucoronide (pNPG) was added to each well as substrate and incubated for a further 30 min at 37ºC. After the incubation period, 100 µL of 0.2 M sodium carbonate was added to terminate the reaction and the absorbance was read at 405 nm.

α-amylase inhibition activity

The α-amylase inhibition method was also carried out according to literature (23). Acarbose was used as a positive control, while phosphate buffer (0.02 M PBS, pH 6.9) was used as a negative control in place of the sample. Each sample was carried out in triplicate with different concentrations. The reaction mixture containing 50 µL of sample solution diluted with buffer, and 25 µL of a-amylase from porcine pancreases (0.5 mg/mL) was incubated for approximately 10 min at 25ºC. Then 50 µL of freshly prepared 0.5% starch solution (w/v) was added to each well as substrate and incubated for a further 10 min at 25ºC. After the incubation period, 100 µL of 1% 3,5-dinitrosalicylic acid (DNS) color reagent was added as a colour reagent and heated in a water bath for 10 min. The absorbance was read at 540 nm.

Antimicrobial activity

Microdilution broth method

In this study, the microdilution broth method was employed for the determination of the minimum inhibitory concentration (MIC) of methanol and water extracts of *P. orientalis* (24). *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa (*ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883) and *Candida albicans* (ATCC 10231) microorganisms were used.

The stock solution was prepared by dissolving the methanol and water extracts in 50% Dimethyl sulfoxide (DMSO) to 50 mg/mL. Mueller Hinton Broth (Accumix® AM1072) for bacteria, and Saboraud Dextrose Broth (HiMedia ME033) for *C. albicans* were used. In the first line of microtiter plates, 90 uL broth was added to the wells and 50 µL aliquots was added to the other wells. The 11th wells were used as sterility controls and 100 µL of broth was added. The 12th wells were used as a reproductive control. 10 µL of extract was added to the first batch of wells and serial dilutions were made. The turbidity of each strain was adjusted to 0.5 McFarland turbidity standard and then diluted in the broth 150 times for bacteria and 1000 times for *C. albicans*. A suspension of 50 µL of microorganism was added to each well to give 5 $x10^5$ CFU/mL for bacteria and 0.5-2.5 $x10^3$ CFU/mL for *C. albicans* (25, 26). The plates with the added bacteria and *C. albicans* were incubated at 37 °C and 35 °C for 16-24 h, respectively. The extract concentration in the wells ranged from 2.5 to 0.004 mg/mL. At the end of the incubation period, in order to observe microbial growth, 50 µL of 2 mg/mL 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) was added to each well and incubated at 37 °C for 2 h. The first wells without colour change were accepted as MIC values. The test was repeated twice and the same results were achieved.

***In-vitro* cytotoxicity assay**

Cell culture

The cytotoxicity of the water and methanol extracts was tested against MDA-MB-231 and L929 cell lines. Both cell lines were cultured in low glucose DMEM containing 10% FBS, 1% L-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin in 25 cm^2 polystyrene flasks and maintained in a humidified atmosphere with 5% CO_2 at 37 °C. Growth and morphology were monitored and cells were passaged when they had reached

85-90% confluence.

Cell proliferation assay

The antiproliferative activity of the extracts was evaluated using the XTT cell proliferation assay against the MDA-MB-231 and L929 cells. Initially, the cancer and control cells were seeded at a density of $5x10^3$ cells per well in 96-well culture plates in 100 µL of culture medium and were allowed to attach overnight before treatment. Then these cells were treated with various concentrations (0.0625, 0.125, 0,25, 0.5, 1 mg/mL) of extracts for 24 h. Following treatments, the medium was removed and wells were washed twice with 200 µL phosphate buffered saline (PBS). At the end of these periods, for determination of living cells, 100 µL DMEM without phenol red and 50 µL XTT labelling mixture were added to each well and then the plates were incubated for another 4 h. The absorbance of XTT-formazan was measured using micro-plate (ELISA) reader at 450 nm against the control (the same cells without any treatment). All experiments were performed in three independent experiments and the cell viability was expressed in % related to control (100% of viability).

Statistical analysis

Data obtained from *in vitro* antioxidant and antidiabetic activity were expressed as the mean ±standard deviation (SD). Cytotoxicity results were evaluated statistically using one-way analysis of variance (ANOVA) at 95% confidence levels for multiple comparisons. The Tukey test was used as the post-hoc test. *P* values less than or equal to 0.05 were considered to be statistically significant. The 50% inhibitory concentration of the extract and reference compounds were calculated through an extract dose-response curve on GraphPad Software (San Diego, CA, USA).

Results and Discussion

Chemical composition

The chemical compositions of the water and methanol extracts of *P. orientalis* were studied using GC-MS (Table 1). According to the GC-MS results, the methanol extract of *P. orientalis* has many more different chemical components. In other words, the methanol solubility of *P. orientalis* leaves is much greater. When the most abundant components in the extracts were examined, for the water extract, 1,4:3,6-Dianhydro-.

Table 1. Chemical components of the water and methanol extracts of *P. orientalis*.

Chemical Components	RT	Relative Content (%)	
		Water	Methanol
Acetic acid, hydroxy-, methyl ester	4.208		0.02
2-Hydroxy-2-cyclopenten-1-one	9.370	4.31	
Perdeuterobenzene	13.358		2.59
4-Chloro-3-methylbut-2-en-1-ol	13.838	0.06	
2,3-Dihydro-3,5-dihydroxy-6-methyl -4H-pyran-4-one	16.711	3.43	
1,2-Benzenediol	18.307	6.78	
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	18.582	7.26	0.49
2-Methoxy-4-vinylphenol	22.461		0.50
1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	25.958	5.69	10.74
Benzene, 1-ethyl-3,5-dimethyl-	26.616		1.08
Benzene, 2-ethyl-1,3-dimethyl-	26.947		1.21
1-Dodecanol (CAS)	28.212		4.53
Phenol, 2,4-bis(1,1-dimethylethyl)-	29.288		2.99
2-Propenoic acid, n-tridecyl ester	33.430		4.73
(-)-Loliolide	35.038		1.53
Hexadecanoic acid, methyl ester	37.699		2.62
Hexadecanoic acid (CAS)	38.346		4.10
Seselin	40.308		0.65
1-Methoxy-1,3-cyclohexadiene	40.417		0.35
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS)	40.526		1.54
Neophytadiene	40.703		7.66
Octadecanoic acid, methyl ester	40.880		0.30
Ethyl linoleolate	41.144		2.81
2H-1-Benzopyran-2-one, 7-methoxy-6-(3-methyl-2-butenyl)	42.351		1.76
Bravelin	42.563		1.26
1-(P-fluorophenyl)-2,3-dimethylnap hthalene	44.016		0.86
3-Keto-isosteviol	45.899		1.52
Kaur-16-en-18-oic acid, 13-hydroxy -, methyl ester, (4.alpha.)-(.+/-.)-	45.990		1.47
1-naphtho[2,1-b]furan-2-yl-2-naphthol	49.938		0.51
Vitamin E	55.557		1.72

alpha.-d-glucopyranose had the highest value, and for the methanol extract 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone. This compound was also the third most common compound in the water extract. Although the methanol extract has many more components, the AChE and BChE inhibition activities and α-amylase inhibition activity of the water extract were higher than in the methanol extract. From this it was concluded that more polar and synergistically more effective components pass to the water extract. However, it should be kept in mind that the methanol extract has more α-glucosidase inhibition activity.

When the contents of the extracts are evaluated in terms of antioxidant activity, generally the methanol extract showed more antioxidant activity for different *in-vitro* systems except for iron chelating activity. This may be due mainly to the greater presence of total phytochemical compounds in the extract of the methanol. When the contents of the extracts were compared according to antimicrobial activity, the methanol extract has more antimicrobial activity than the water extract. Probably the main reason for this is that more total phytochemical compounds are present in the methanol extract.

Enzyme activity

The methanol and water extracts obtained from *P. orientalis* leaves were evaluated for their inhibitory effects against the Alzheimer's disease-related enzymes, AChE and BChE, diabetes mellitus-related enzyme α-glucosidase and α-amylase (Table 2). The water extract showed stronger AChE and BChE inhibition activity than the methanol extract. Similarly, α-amylase inhibition activity was also higher than in the methanol extract. However, the α-glucosidase activity of methanol extract was stronger than in the water extract. It should be emphasized that neither the water nor the methanol extract demonstrated more α-glucosidase and α-amylase inhibition activity than the antidiabetic drug-acarbose at the same concentration level.

Antioxidant activity

The methanol and water extracts prepared from *P. orientalis* leaves were evaluated for antioxidant activity by different *in-vitro* systems, including DPPH, ABTS radical scavenging, total phenol, total flavonoid, iron chelating and ferric reducing power assay methods. The results are presented in Figure 1.

The total phenolic content of the extracts was expressed as milligrams of gallic acid (phenolic content: 13.85±0.78 for water extract and 15.35±0.28 for methanol extract) equivalent per gram of *P. orientalis* leaves.

The methanol extract showed higher DPPH radical scavenging activity than the water extract, while both extracts showed the same activity in ABTS radical scavenging. In the DPPH and ABTS radical scavenging assay, the water and methanol extracts demonstrated scavenging activity in a concentration-dependent manner. However, at lower concentrations, the methanol extract is more active than the water extracts, while the concentration did not have much effect on ABTS radical scavenging with both of the extracts.

Since metal ions in biological systems play an inductive role in the formation of free radicals, the ability of methanol and water extracts from plane leaves to form chelate with metal was investigated in this study. The results demonstrated that, the water extract exhibited strong iron chelating activity, which may be related to the polar compounds present in the water extract at higher amounts than in the methanol extract.

Overall, it was seen that the *in-vitro* antioxidant and antidiabetic activity was linked with the phytochemical compounds such as total phenolic and flavonoid present in the extracts. It is noteworthy that the methanol and water extracts exhibited strong α-glucosidase and α-amylase inhibition activity, suggestings that other phytochemical compounds (alkaloids, terpenes, lignans etc.) may contribute to the enzyme activity. Therefore, further bioassay-guided phytochemical and active compound identification studies are required to determine potential antioxidant and antidiabetic therapeutic lead compounds.

Antimicrobial activity

The antimicrobial activities of *P. orientalis* extracts against 5 bacteria and *Candida albicans* were detected

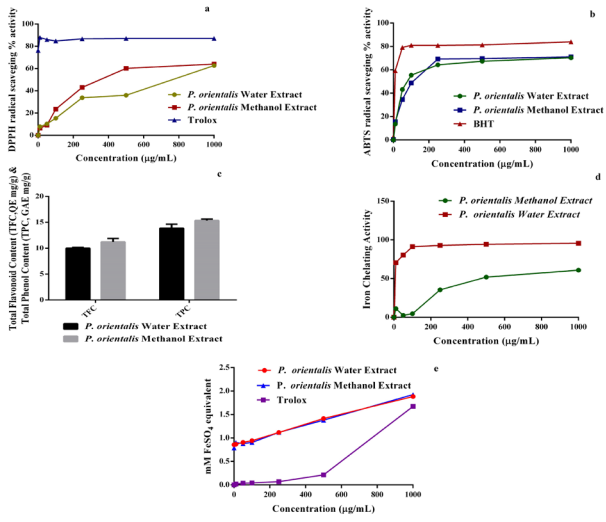

Figure 1. The *in-vitro* antioxidant activity of the methanol and water extracts of *P. orientalis* leaves; a) DPPH radical scavenging activity; b) ABTS radical scavenging activity; c) total phenol and total flavonoid contents; d) iron chelating activity; e) ferric reducing power as $FeSO_4$ equivalent.

Table 2. Enzyme inhibitory activities (%) of the water and methanol extracts of *P. orientalis* leaves (at 2 mg/mL concentrations).

Extracts	Anticholinesterase activity		Antidiabetic activity	
	AChE	BChE	α-glucosidase	α-amylase
Water	65.61 ± 4.12	79.51 ± 0.97	62.50 ± 2.91	75.29 ± 0.68
Methanol	54.39 ± 3.19	65.76 ± 4.04	63.04 ± 0.47	69.06 ± 2.20
Reference Drugs				
Galanthamine hydrobromide	93.87± 0.56	89.89 ± 0.01		
Acarbose			57.56 ± 0.52	58.40 ± 0.63

Table 3. Antimicrobial activity results of *P. orientalis* extracts.

Extracts	Microorganisms and MIC Values (mg/mL)					
	E. coli **ATCC 25922**	***S. aureus*** **ATCC 29213**	***P. aeruginosa*** **ATCC 27853**	***E. faecalis*** **ATCC 29212**	***K. pneumoniae*** **ATCC 13883**	***C. albicans*** **ATCC 10231**
Water	2.5	0.156	>2.5	>2.5	>2.5	>2.5
Methanol	1.25	0.018	0.625	1.25	>2.5	1.25

in the range of 2.5 to 0.018 mg/mL (Table 3). It has been reported that antimicrobial activity of plant extracts is significant if the MIC value is 0.1 mg/mL or less, moderate if the MIC value is in the range of 0.1 < MIC ≤ 0.625 mg/mL and weak if the MIC value is greater than 0.625 mg/mL (27, 28). Based on these criteria, the water extracts obtained from *P. orientalis* were found to be moderate (0.156 mg/mL) on the *Staphylococcus aureus*, and weak on the other tested bacteria and *C. albicans*. However, methanol extracts were determined to be more effective on bacteria and fungi than the water extracts. The methanol extracts of *P. orientalis* were found to be effective (0.018 mg/mL) on S. aureus, and moderate (0.625 mg/mL) on *P. aeruginosa*. However, the water and methanol extracts of *P. orientalis* were detected to have weak antimicrobial effects (>0.625 mg/mL) on the other microorganisms.

Cell viability

The *in-vitro* cytotoxicity of water and methanol extracts of *P. orientalis* was evaluated both on MDA-MB-231 and L929 cell lines with the XTT assay and the results are given in Figure 2. According to the experimental results, MDA-MB-231 cell proliferation reduced dramatically ($p<0.05$) in a dose-dependent manner in the presence of water and methanol extracts at all concentrations when compared with the control group. Moreover, the methanol extracts showed more anti-proliferative effects than the water extracts. This may be due to the fact that the methanol extract has a richer chemical content than the water extract, as shown in Table 1. The IC_{50} of water and methanol extracts of *P. orientalis* in MDA-MB-231 cell lines was calculated as 0.053 mg/mL and <0.0625 mg/mL, respectively. However, neither extract showed any significant cytotoxicity on the L929 cell line at the IC_{50} concentrations. In general, the results suggest that *P. orientalis* extracts may have potent anti-proliferative activity against MDA-MB-231 cell lines and may be useful as anticancer agents. Nevertheless, further studies are warranted to investigate the anticancer properties of *P. orientalis*.

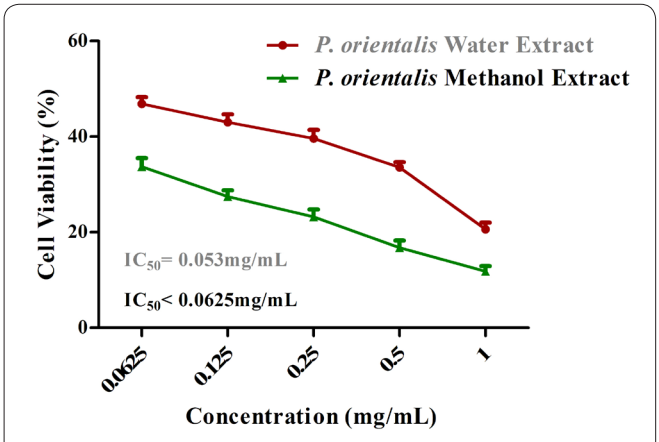

Figure 2. Effects of water and methanol extracts from *P. orientalis* on the viability of MDA-MB-231 cell line, after treatment with different concentrations (range: 0.065-1 mg/mL) for 24 h.

In summary, the results of this study showed the biological activity of water and methanol extracts of *P. orientalis*. The chemical content of the extracts was clarified by GC-MS. The results of this study showed that both extracts have good inhibitory effects against AChE, BuChE, α-glucosidase and α-amylase enzymes. It is necessary to emphasize that both methanol and water extracts demonstrated more α-glucosidase and α-amylase inhibition activity than the antidiabetic drug-acarbose at the same concentration level. The antioxidant, anticancer and antimicrobial results of the leaf extracts can be seen as highly promising. In conclusion, these results indicate that the various extracts of *P. orientalis* L. leaves may be useful in preparing pharmaceutical formulations to treat various diseases such as diabetes, cancer, Alzheimer's disease etc. In this respect, further studies are being carried out in our laboratory.

References

1. Zencirkiran M, Erken K. The Effect of Different Times Collecting Cutting and Auxin Treatments of The Rooting in *Platanusorientalis* L. (Oriental plane tree). J Anim Plant Sci 2012; 22:764–767.

2. Pourkhabbaz A, Rastin N, Olbrich A, Langenfeld HR, Polle A. Influence of environmental pollution on leaf properties of urban plane trees, *Platanus orientalis* L. Bull Environ Contam Toxicol 2010; 85: 251–255.

3. Hajhashemi V, Ghannadi A, Mousavi S. Antinociceptive study of extracts of *Platanus orientalis* leaves in mice. Res Pharm Sci 2011; 2:123–128.

4. Mitrokotsa D, Mitaku S, Demetzos C, Harvala C, Mentis A, Perez S, Kokkinopoulos D. Bioactive compounds from the buds of *Platanus orientalis* and isolation of a new kaempferol glycoside. Planta Med 1993; 59:517–520.

5. Mitrocotsa D, Bosch S, Mitaku S, Dimas C, Skaltsounis AL, Harvala C, Briand G, Roussakis C. Cytotoxicity against human leukemic cell lines and the activity on the expression of resistance genes of flavonoids from *Platanusorientalis*. Anticancer Res 1999; 19:2085–2088.

6. Ibrahim MA, Mansoor AA, Gross A, Ashfaq MK, Jacob M, Khan SI, Hamann MT. Methicillin-resistant Staphylococcus aureus (MRSA)-active metabolites from *Platanus occidentalius* (American sycamore). J Nat Prod. 2009; 72:2141–2144.

7. Zargari A. Medicinal plants, 6th ed., Tehran University Publications, Tehran, 1990, pp. 469–470.

8. Kazemi N, Kahrizi D, Mansouri M, Karim H, Vaziri S, Zargooshi J, Khanahmadi M, Shokrinia M, Mohammadi N. Induction of linalool as a pharmaceutical and medicinal metabolite via cell suspension culture of cumin (*Cuminum cyminum L.*). Cell Mol Biol 2016; 62: 65-68

9. Pohanka M. Cholinesterases, A Target Of Pharmacology And

Toxicology. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2011; 155:219–230.
10. Giacobini E. Cholinesterases: New Roles in Brain Function and in Alzheimer's Disease. Neurochem Res 2003; 28:515-22.
11. Ali H, Houghtona PJ, Soumyanathb A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthusamarus*. J Ethnopharmacology 2006; 3:449-455.
12. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergeniaciliata* Haw.). Food Chem 2008; 1:247-252.
13. Jian B, Zhang H, Han C, Liu J. Anti-Cancer Activities of Diterpenoids Derived from *Euphorbia fischeriana* Steud. Molecules 2018; 23:1-11.
14. Grollino MG, Raschellà G, Cordelli E, Villani P, Pieraccioli M, Paximadas I, Malandrino S, Bonassi S, Pacchierotti F. Cytotoxicity, genotoxicity and gene expression changes elicited by exposure of human hepatic cells to *Ginkgo biloba* leaf extract. Food Chem Toxicol 2017; 109:486-496.
15. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food chemistry 2005; 91:621-632.
16. Clarke G, Ting KN, Wiart C, Fry J. High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the Malaysian Rainforest. Antioxidants 2013; 2:1-10.
17. Chun SS, Vattem DA, Lin YT, Shetty K. Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against Helicobacter pylori. Process Biochem 2005; 40:809-816.
18. Yang H, Dong Y, Du H, Shi H, Peng Y, Li X. Antioxidant Compounds from Propolis Collected in Anhui, China. Molecules 2011; 16:3444-3455.
19. Chai TT, Mohan M, Ong HC, Wong FC. Antioxidant, iron-chelating and anti-glucosidase activities of *Typha domingensis* Pers (Typhaceae). Trop J Pharm Res 2014; 13:67–72.
20. Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem 2007; 105:940–949.
21. Ellman GL, Courtney KD, Andres V, Featherstone RM. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochem Pharmacol 1961; 7:88–95.
22. Kumar D, Kumar H, Vedasiromoni JR, Pal BC. Bio-assay guided isolation of α-glucosidase inhibitory constituents from *Hibiscus mutabilis* leaves. Phytochem Anal 2012; 23:421–425.
23. Kumar D, Gupta N, Ghosh R, Gaonkar RH, Pal BC. α-Glucosidase and α-amylase inhibitory constituent of *Carex baccans*: Bio-assay guided isolation and quantification by validated RP-HPLC-DAD. J Funct Foods 2013; 5:211–218.
24. Eloff JN, A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 1998; 64:711–713.
25. CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.
26. CLSI, Reference Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, 2nd ed., NCCLS document M27- A2. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087- 1898, USA, 2002.
27. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med 2010; 76:1479–1491.
28. Awouafack MD, McGaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M, Eloff JN. Antimicrobial Activity and Cytotoxicity of the Ethanol Extract, Fractions and Eight Compounds Isolated from *Eriosemarobustum* (Faba-ceae). BMC Complement Altern Med 2013; 13:289-297.