Ind. J. Pharm. Edu. Res, 2023; 57(2):511-518.
https://www.ijper.org

Original Article

New Data for Endemic *Phlomis cypria* Post from North Cyprus: Biological Activities and LC MS/MS Analysis

İmge Kunter[1], Mohammed Tamim Tarabishi[1], Niloufar Zabib[1], Tuğba Erçetin[1], Mehmet İlktaç[1], Fatih Göger[2,3], Müberra Koşar[1,*]

[1]Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, North Cyprus via Mersin, TURKEY.
[2]Faculty of Pharmacy, Department of Pharmacognosy, Tepebaşı, Eskişehir, TURKEY.
[3]Department of Pharmacy, Program in Pharmacy Services, Yunus Emre Vocational School, Anadolu University, Tepebaşı, Eskişehir, TURKEY.

ABSTRACT

Background: The genus *Phlomis* includes up to 100 species which are distributed throughout Eurasia. Of these species, *Phlomis cypria* Post (Lamiaceae) is an endemic plant from North Cyprus. *P. cypria* is commonly used in North Cyprus as medicinal herbal tea for the winter cold and flu. *Phlomis* species have antioxidant, antimicrobial and anticancer effects. **Materials and Methods:** In this study, herbal part of *P. cypria* from North Cyprus was sequentially extracted using hexane, dichloromethane and 70% aqueous methanol and using maceration at room temperature. All extracts were investigated for antiradical (DPPH$^{\bullet}$ and ABTS$^{+\bullet}$), anticholinesterase, antibacterial and the cytotoxic activities and additionally, chemical compositions of the extracts were evaluated by LC MS/MS method. **Results:** Aqueous methanol extract showed antiradical activities (IC_{50} 0.29 mg/mL in DPPH$^{\bullet}$; TEAC 1.22, 1.36 in ABTS$^{+\bullet}$) and cytotoxic activities (IC_{50} 1264 µg/mL on SK-HEP 1 cells). Dichloromethane extract only showed activity in anticholinesterase assay (inhibitions were 37.68% for AChE; 21.73% for BChE). Forsytoside B was found as the main phenolic compound in the active extract with caffeic acid bound to other compounds. **Conclusion:** According to the results, *P. cypria* could be a potential candidate as cytotoxic and cholineasterase inhibitor agent.

Keywords: *Phlomis cypria*, Lamiaceae, Antioxidant, Cytotoxicity, Antibacterial, Anticholinesterase.

Correspondence:
Prof. Dr. Müberra Koşar
Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, North Cyprus via Mersin-10, TURKEY.
Email id: muberra.kosar@emu.edu.tr

Received: 18-12-2021;
Revised: 28-03-2022;
Accepted: 24-06-2022.

INTRODUCTION

Many Lamiaceae plants such as basil, mint, sage and lavender are widely used in some food preparations because of their aromatic properties. *Phlomis* is a genus of herbaceous flowering shrub in the Lamiaceae family.[1] There are over 100 *Phlomis* species. While they are native to the Mediterranean regions, they cover most of Asia as far as China. They are widely used for their pharmacological activities.[2] In Bangladesh, the leaves of *P. aspera* (Willd.) are used to help with the treatment of psoriasis and chronic skin diseases.[3] The flowers are traditionally used along with honey as cough treatment and in fighting colds in children. In different countries, various *Phlomis* species have been established to be used for homeopathy for skin ailments, wounds, lesions, burns irritations and scalds in the form of plasters prepared from freshly chopped leaves.[4-9] Along with other plants, the aerial parts of *P. bracteosa* Royle ex Benth. is used in China as powder for gastric disorders, too.[4] Some herbal teas including *Phlomis* sp. are also used as stimulants and appetizers. Studies had emerged confirming the efficacy of *Phlomis* sp. for the management of ulcers and hemorrhoids. Additionally, as well as anti-inflammatory activities, researches have confirmed antiradical and antimicrobial activities against several types of bacteria.[9]

Phlomis sp. contain essential oils, flavonoids, iridoids and phenylethyl alcohol glycosides as major classes of secondary metabolites.[9] *Phlomis* essential oils are rich in monoterpenes, sesquiterpenes, acids, alcohols and aliphatic compounds according to the growing areas. Similar to other Lamiaceae plants, essential oil configuration of *Phlomis* species can be used for their chemotaxonomic classifications.[10-13] Flavonoids, which are the main phytoconstituents isolated from the *Phlomis* genus, are the responsible constituents from many of the biological activities. Compounds such as apigenin, luteolin, naringenin, eriodictyol, chryseriol and kaempferol are plentiful. 7-*O*-glycosides of the mentioned flavonoids are present in most of the *Phlomis* sp. in varying concentrations.[14] Although, they are not the major compounds, various iridoids have been isolated and identified from *Phlomis* sp. Commonly, shanzhiside methyl ester and its derivatives and new iridoid, phlomiol and phlorigidosides, have been discovered.[15] Another chemical group which is

DOI: 10.5530/ijper.57.2.62

commonly found in high concentrations in the *Phlomis* sp. is the phenylethylalcohol glycosides. Compounds such as verbascoide and forsythosides have been discovered in a couple of *Phlomis* sp. and caffeic acid bound constituents are also identified in the *Phlomis* species. In recent years, many new phenylathylalcohol glycosides such as phlinosides, samioside and alyssonoside have been discovered from various *Phlomis* sp.[16]

Cyprus is a small island in the Mediterranean Sea; however with more than 1500 species, it is abundant with flowers and shrubs. The number of endemic species which are found in Cyprus has been determined to be around 121.[17] *Phlomis cypria* Post., an endemic plant of North Cyprus, has complete opposite and decussate leaves with wrinkled surface and yellow Lamiaceae type of flowers. *P. cypria* is naturally distributed around the Saint Hilarion peak and Alev Kayası (Fire Rock) region in North Cyprus.[17] Because of its endemism, there is no detailed scientific research regarding the chemical composition and biological activities of *P. cypria* in the literature. *P. cypria* is commonly used in north Cyprus as herbal tea. Other uses include the homeopathic treatments of diabetes, fever and others for powdered leaves.

The aim of this research project was to screen the main pharmacological activities such as antiradical, anticholinesterase, antibacterial activities and the cytotoxic ability of the polar extract with phenolics as well.

MATERIALS AND METHODS

Plant Material and Reagents

Aerial parts of *Phlomis cypria* Post. were collected from Girne, Saint Hilarion Castle area (April 15, 2017). The samples were identified by Prof. Dr. F. Neriman Ozhatay from the Faculty of Pharmacy Eastern Mediterranean University. Chromatographic standards were purchased from Sigma Chemical Company (St. Louis, MO, USA). All remaining reagents were of the highest purity available and obtained from the Sigma Chemical Company (St. Louis, MO, USA).

Preparation of the Extracts

Air dried *P. cypria* herbal parts (77 g) were powdered and successively extracted with 500 mL of hexane, dichloromethane and 70% aqueous methanol. After 24 hrs, the solvent was filtrated and the extraction procedure was repeated 2 more times with the same solvent. Combined extracts were evaporated under vacuum at 40°C. The extracts were lyophilized and stored at 4°C until further analyses.

Total Phenolics

Total phenols were estimated as gallic acid equivalents (GAE), expressed as mg of gallic acid/g extract.[18] First, 100 µl of sample was transferred to a 10 mL volumetric flask 6 mL of water, to which 500 µl of undiluted Folin-ciocalteau reagent was subsequently added. After 1 min, 1.5 mL of 20% aqueous sodium hydrogen carbonate was added and the volume was raised up to 10.0 mL with water. The controls contained all the reaction reagents except for the actual extract. Following 30 min of incubation at 25°C, the absorbance was measured at 760nm and compared to a gallic acid calibration curve. Total phenolics were determined as gallic acid equivalents and were presented as the mean of triplicate analyses. The experiment was repeated at 2 concentrations for each extract.

LC MS/MS Analysis

LC MS/MS analysis was carried out using an Absciex 3200 Q trap MS/MS detector. Experiments were performed using a Shimadzu 20A HPLC system coupled to an Applied Biosystems 3200 Q-Trap LC-MS/MS instrument equipped with an ESI source operating in negative ion mode. For the chromatographic separation, a GL Science Intersil ODS 250 × 4.6 mm, i.d., 5µm particle size, octadecyl silica gel analytical column operating at 40°C has been used. The solvent flow rate was maintained at 0.5 mL/min. Detection was carried out with a PDA detector. The elution gradient consisted of mobile phases (A) acetonitrile: water: formic acid (10:89:1, *v/v/v*) and (B) acetonitrile: water: formic acid (89:10:1, *v/v/v*). The composition of B was increased from 10% to 100% in 40 min. LC-ESI-MS/MS data were collected and processed by Analyst 1.6 software.

1,1-Diphenyl-2-picrylhydrazyl (DPPH•) Radical Scavenging Activity

The ability of *P. cypria* to scavenge DPPH• free radicals was determined by the method of Gyamfi *et al.* (1999).[19] Briefly, a 50 µl aliquot of each extract in Tris-HCl buffer (50 mM, pH-7.4) was mixed with 450 µl of Tris-HCl buffer (50mM, pH 7.4) and 1 mL of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl in methanol. The controls contained all the reaction constituents excluding the extract or positive control material. After 30 mins of incubation in dark and at ambient temperature (25°C), the subsequent absorbance was documented at 517 nm. The percentage inhibition was calculated using Equation 1. BHT was used as a positive control. The values were presented as the average of three measurements.

$$\text{Percentage inhibition} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100 \quad \text{(Eq. 1)}$$

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS$^{+\bullet}$ radical scavenging activity

The ability of *P. cypria* to scavenge ABTS$^{+\bullet}$ free radicals was determined by the method of Re *et al.* (1998).[20] ABTS$^{+\bullet}$ solution was prepared by adding 36 mg of ABTS$^{+\bullet}$ along with 6.6 mg of $K_2O_8S_2$ in 10 mL of distilled water. This solution was then left in dark for 16 hrs. The absorbance was adjusted between 0.7-0.8 at 734 nm. The samples were dissolved in the concentration 3 mg/mL and 6 mg/mL. 10 µl of samples was added to 990 µl of ABTS$^{+\bullet}$ reagent. The experiment was repeated 3 times at each

concentration. The results were presented as an equivalent value of the antioxidant capability of Trolox.

Antimicrobial Activity

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 700603) were used in this assay. The antimicrobial activities of the extracts were evaluated by the broth dilution method. Briefly, the bacteria were cultured overnight in an incubator at 37°C in a cation supplemented Mueller-Hinton broth. The dichloromethane and hexane extracts were prepared at 2048 mg/l in dimethyl sulfoxide (DMSO) as stock solutions. Seventy percent aqueous methanol extract was prepared in distilled water at a concentration of 2048 mg/l for stock solution. The experiments were carried out at concentration of 64mg/L for each dichloromethane and hexane extracts. This was done to minimize the concentration of DMSO to 3%, preventing the results from being influenced by its antimicrobial activity. The 70% aqueous methanol extract was carried out at 1024 mg/L. Minimum inhibitory concentration was regarded as the minimum concentration of the extract that inhibits the growth of bacteria. The experiments were repeated three times and an average was obtained.

Cholinesterase Inhibition Activity

Acetylcholine esterase (AChE) and buthyrylcholine esterase (BChE) inhibition processes were carried out by a small modification to the spectrophotometric method explained by Ellman *et al.* (1961).[21] *Electric eel* AChE (Type VI-S, EC3.1.1.7) and horse serum BChE (EC3.1.1.8) were applied, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. 5,5'-Dithio-bis(2-nitrobenzoic) acid (DTNB) was used for the measurement of the anticholinesterase activity. This procedure had 168 µl of 50 mM of Tris HCl buffer (pH 8.0), 2 µl of each sample solution, 10 µl of 6.8 mM DTNB solution containing 20 mM $MgCl_2$ and 100 mM NaCl, 10 µl of AChE/ BChE solution were added by a multichannel automatic pipette. The well plate used had 96 wells and it was incubated at 25°C for 15 min. The process was then commenced by the addition of 10µl of acetylthiocholine iodide/butyrylthiocholine chloride. The propagation of the reaction in which acetylthiocholine iodide/butyrylthiocholine chloride was being hydrolyzed was monitored by the materialization and appearance of the yellow 5-thio-2-nitrobenzoate anion as a consequence of the reaction between DTNB and thiocholines which was catalyzed by the enzymes at wavelength of 412 nm. The percentage of inhibition of the enzymes was concluded by comparison to the blank samples which contained ethanol in the phosphate buffer solution using the Eq 2. The experiments were done in triplicates and Donepezil was used as a reference.

$$\% \text{ Inhibition} = (E-S)/E \times 100 \qquad \text{(Eq 2)}$$

E =activity of enzyme without test sample

S = activity of enzyme test sample

Cell culture and MTT Assay

Human Hepatic Adenocarcinoma was kindly provided by Prof. Dr. Mehmet Öztürk (Izmir Biomedicine and Genome Centre). The cells were kept in an enriched DMEM solution containing 10% FBS, 100 U/mL penicillin, 2 mM L-glutamine, 100 mg/mL streptomycin and 1X NEAA under a humidified medium at 5% CO_2 at an ambient temperature of 37°C. The MTT assay was carried out in order to analyze the cytotoxic effect of *P. cypria*'s 70% aqueous methanol extract. Concentrations ranging from 100 to 1500 µg/mL *P. cypria* aqueous methanolic extract were used on SK-HEP-1 cancer cell line.

$2x10^3$ cells were seeded in a 96 well plate. After an overnight incubation, cells were subsequently incubated with the varying concentrations of the 70% methanol extract. After 48 hrs incubation, 0.5 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added for 4 hrs. Following the medium removal, the formazan crystals were dissolved with 100 µl DMSO. The absorption of the solution was measured at 570 nm using a Thermo Varioscan microplate reader. The total cell viability was calculated as viable cell percentage. The experiments were repeated three times and an average was obtained.

Statistical Analysis

Experiments have been independently carried out three times and all results were presented as mean ± SD from three measurements for cytotoxicity analysis, Acetylcholine/ butyrylcholine esterase activity, and antioxidant assay. For the cytotoxicity assay One-way ANOVA test was used for analysis. $P < 0.05$ was considered as statistically significant. IC_{50} (the median growth inhibitory concentration) values have been calculated from the dose response inhibition curve. All the analyses were performed using GraphPad Prism 5 software. LC-ESI-MS/MS data were collected and processed by Analyst 1.6 software.

RESULTS

Chemical Composition Analysis

The herb parts of *Phlomis cypria* collected from Northern Cyprus were roughly shredded and then macerated with hexane, dichloromethane and 70% methanol at room temperature, respectively. The total phenolic constituents evaluated by spectrophotometric method using Folin-Ciocalteau reagent in two different concentrations. The extract yields and total phenol amounts are given in Table 1. According to our results, the highest yield was obtained from aqueous methanol extract and the total phenol content of the methanolic extract was also found to be the highest among the others.

Table 1: Extract yields and total phenolics of *Phlomis cypria* extracts.

Extract	Extract Yield (%)	Concentration (mg/mL)	Total phenols (mg_{GAE}/g_{ext})
70% Methanol	29.36	3	8.83 ± 0.34*
		6	3.76 ± 0.07
Hexane	4.84	3	0.14 ± 0.20
		6	1.74 ± 1.78
Dichloromethane	9.68	3	1.50 ± 0.31
		6	1.43 ± 0.35

*mean ± SD (n=3)

Table 2: LC MS/MS analysis of *Phlomis cypria*.

No	RT	$[M-H]^-$	MS^2	Identified as
1.	3.5	191	173, 127	Quinic acid.
2.	7.0	353	335, 191, 179, 161, 135	5-Caffeoylquinic acid
3.	7.4	755	623, 593, 461, 179, 161, 135	Forsytoside B
4.	8.8	623	461	Verbascoside
5.	10.0	477	315, 300	Isorhamnetin glucoside
6.	11.7	461	299, 283, 269, 255	Chrysoeriol glucoside
7.	12.9	445	269, 175, 113	Apigenin glucuronide
8.	13.3	474	299, 284	Chrysoeriol glucuronide
9.	19.4	299	284, 255	Chrysoeriol

In this study, the chemical composition of the methanol extract of *P. cypria* which showed high effect in most of the biological activity studies was analyzed by LC MS/MS method. Polar extracts were fragmented in negative mode and compared with literature data and estimated compound analysis was performed. The results of this evaluation are given in Table 2 (Figure 1).

Antiradical Activities

70% aqueous methanol extract was able to scavenge $DPPH^{\bullet}$ in a dose dependent manner as presented in the sigmoid curve of the percentage inhibition versus the log of the concentration in Figure 2. The IC_{50} value of aqueous methanol extract was calculated as 0.29 mg/mL using the sigmoidal curve as well.

The ability of the three extracts of *P. cypria* to scavenge $ABTS^{+\bullet}$ radical at two different concentrations of 3 and 6 mg/mL were measured. The results of the extracts are given in Figure 3 as TEAC value. The $ABTS^{+\bullet}$ results shown are a representation of the three extracts in terms of their equivalent values of Trolox. The 70% methanol extract was shown the maximum inhibitory activity when compared with the dichloromethane and hexane extracts. Higher TEAC value indicates a higher scavenging activity of the $ABTS^{+\bullet}$ radical.

Antimicrobial and Cholinesterase Inhibition Activities

The results of antimicrobial activities of the extracts against the tested microorganisms were not statistically different. Only aqueous methanol extract of *P. cypria* has shown a very weak antimicrobial activity with the MIC ≥ 128 mg/L.

The extracts were analyzed for their acetylcholineesterase inhibitory activities and donepezil was used as a reference standard. The results of the extracts are given in Table 3 as percentages. According to the results within the Table 3, the extracts were shown more inhibition against the acetylcholine esterase than the butyrylcholineesterase enzymes. The highest activity (37.68%) within the extracts was obtained from dichloromethane extract as moderate acetylcholine esterase inhibition in *in vitro* assay.

Cell culture and MTT assay

The cytotoxic effects of the extracts of *P. cypria* were investigated on SK-HEP-1 cell line by *in vitro* assay. Hexane and dichloromethane extracts have not shown any cytotoxic activities on the SK-HEP-1 cells maybe because of solubility problems in polar media. Cytotoxic effect of aqueous methanol extract of *P. cypria* significantly increased in a concentration-dependent manner. At concentrations over 500 µg/mL, the cytotoxicity increased drastically as shown in Figure 4 ($P < 0.001$). The IC_{50}

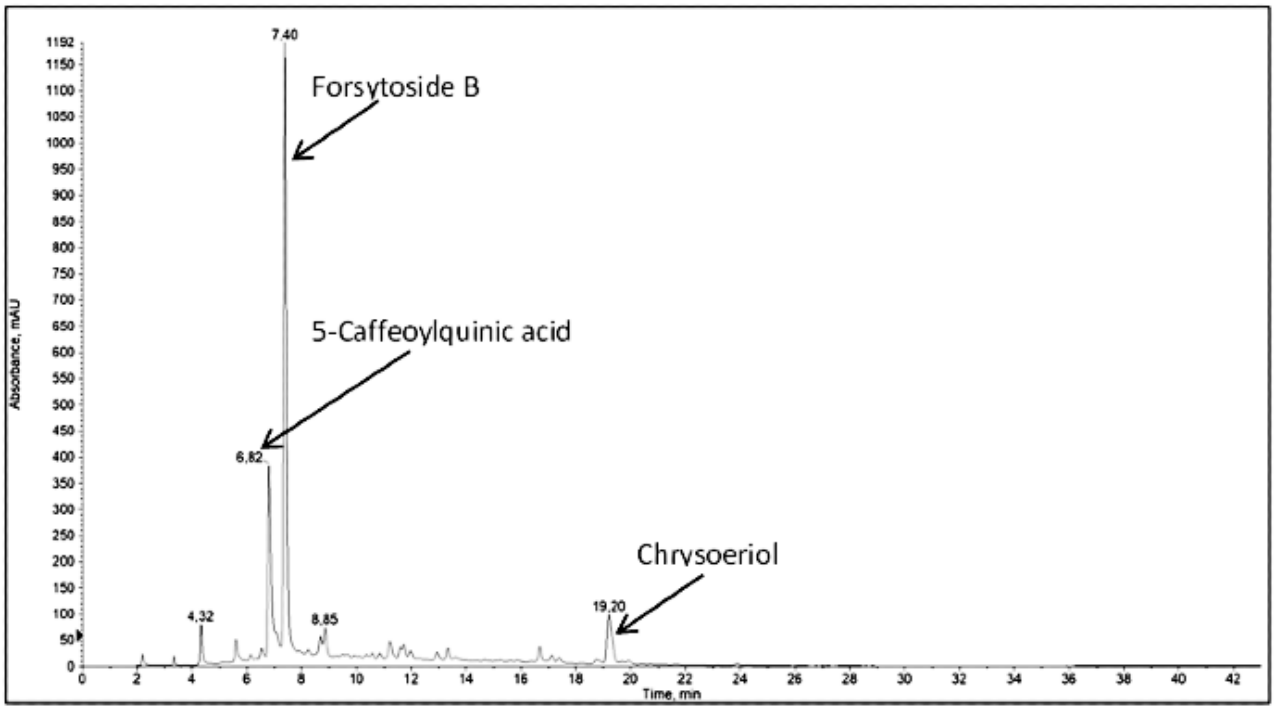

Figure 1: LC chromatogram of aqueous methanol extract of *P. cypria* at 330 nm.

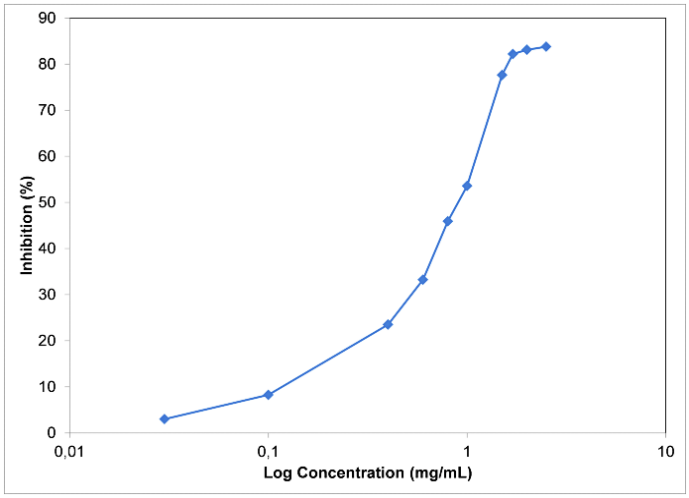

Figure 2: Sigmoidal inhibition graph of DPPH• radical with *P. cypria*.

value of aqueous methanol extract of *P. cypria* was determined as 1264 µg/mL.

DISCUSSION

In the literature, other *Phlomis* species were investigated and published by different research groups. Especially, essential oils compositions and their activities of *Phlomis* species were evaluated. On the other hand, phytochemical studies included iridoids and flavonoids also found in the literature for many *Phlomis* species.

In this study, some biological activities and chemical composition of different extracts of herb part of endemic *P. cypria* were investigated. This research is the first screening study of biological activities of *P. cypria* and as well as its chemical composition. Therefore, it was not possible to compare the results of this study with the literature for *P. cypria* taxa.

According to the LC MS/MS analysis phenolic compounds were identified in the active fraction. The compounds within the polar active extract were evaluated as follows:

Compound 2 presented molecular ion at *m/z* 353 which was fragmented at *m/z* 191 (quinic acid) as a base peak and *m/z* 179 (approximately 5% intensity of the base peak). Loss of 62 amu between the molecular ion peak and the base peak ion indicates a caffeoyl or hexose unit but the presence of ion at *m/z* 135 indicated a caffeic acid moiety. According to the previously published data, compound 2 was identified as 5-caffeoylquinic acid. Quinic acid was also identified in the extract as compound 1.[22]

Compound 3 showed molecular ion peak at *m/z* 755 [M-H]-, due to the loss of a pentose sugar (-132 amu) and caffeic acid loss (-162 amu) *m/z* 623 and 593 were observed respectively. The combined loss of these units produced the ion at *m/z* 461. Caffeic acid fragments were also observed at *m/z* 179, 161 and 135. Compound 3 was identified as forsythoside B (Figure 5) which is common in *Phlomis* species.[23] Compound 4 showed a molecular ion peak at *m/z* 623 [M-H]-, then fragmented to ion at m/z 461 due to the loss of a caffeoyl moiety. The fragmentation pattern matches with verbascoside which is common in *Phlomis* genus.[24]

Compound 5 showed a molecular ion peak at *m/z* 477 [M-H] and a base peak at *m/z* 315 due to the loss of a glucose moiety. Other fragments of ions were observed at *m/z* 299. According to the previously publish LC-MS literature data about *Phlomis* phenolics, the compound was tentatively identified as isorhamnetin glucoside.[25]

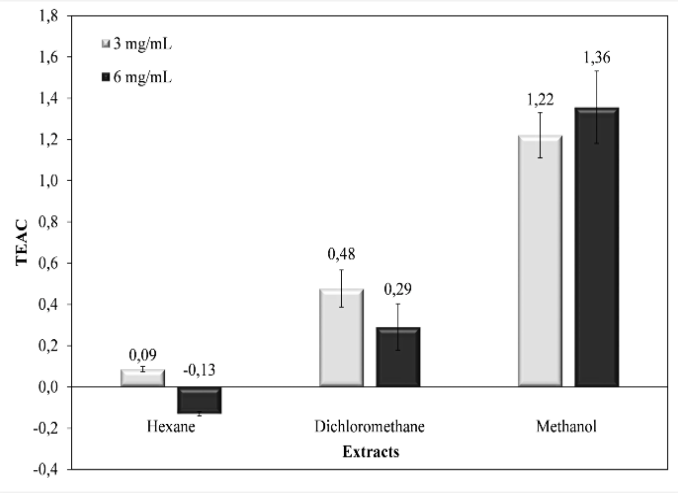

Figure 3: TEAC values of *P. cypria* extracts. Data represent the mean values of three experiments (± SD).

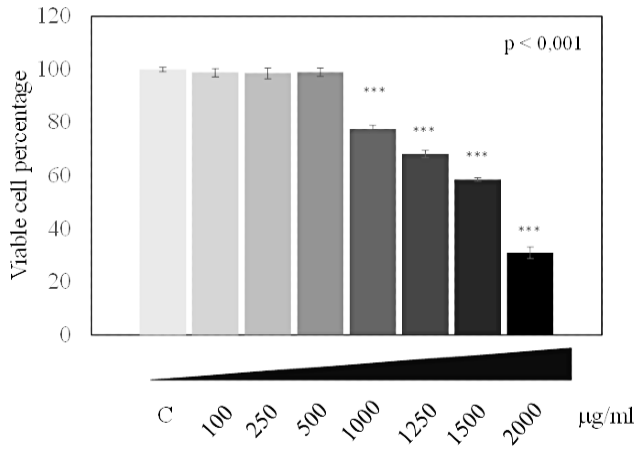

Figure 4: Cell viability effects of aqueous methanol extract of *P. cypria* on SK-HEP 1 cell line. Data represent the mean values of three individual experiments (± SD). *** $P < 0.001$.

Table 3: Acetylcholine and butyrylcholine esterase activities of *Phlomis cypria* extracts.

Sample	Concentration (mg/mL)	AChE activity (%)	BChE activity (%)
H*	0.5	11.88 ± 0.16**	9.35 ± 0.02
H	1.0	17.05 ± 0.07	13.47 ±0.02
H	2.0	22.79 ± 0.09	19.54 ±0.01
C	0.5	19.05 ± 0.05	13.28 ±0.04
C	1.0	27.79 ± 0.06	18.80 ± 0.02
C	2.0	37.68 ± 0.08	21.73 ± 0.04
M	0.5	4.25 ± 0.04	10.96 ± 0.01
M	1.0	6.78 ± 0.06	13.85 ± 0.01
M	2.0	9.92 ± 0.04	17.67 ± 0.02
Donepezil		99.85 ± 0.01	69.42 ± 0.04

*H, hexane extract; C, dichloromethane extract; M, methanol extract; **mean ± SD (n=3)

Compound 6 showed a molecular ion peak at *m/z* 461 which was 162 amu (glucose) higher than chrysoeriol. Other fragments of compound 4 was observed at *m/z* 299 (chrysoeriol) and 255. So compound 6 was identified as chrysoeriol glucoside. Compund 6 was identified as chrysoeriol glucuronide which was presented 176 amu (glucuronic acid) higher molecular ion peak than chrysoeriol.

Compound 7 showed a molecular ion at *m/z* 445 [M–H]- and a base peak ion at *m/z* 269 due to the loss of a glucuronic acid moiety. MS2 fragmentation of aglycon produced ions at *m/z* 175 and 113. Comparing these data with the literature published previously, compound 7 was identified as apigenin glucuronide.[26]

Compound 9, presented molecular ion at *m/z* 299 and base peak ion at *m/z* 284, owing to the loss of a methyl unit, was identified as chrysoeriol which was previously isolated and identified in the same species.[27] Chrysoeriol glucuronide also identified as compound 8 using *m/z* 299, 284 MS^2 fragments.[26]

Degradation by oxidative compounds of chemicals and organelles present in cells is a multiphase reaction requiring several initiation steps and propagations. The core concept to block oxidative degradation from occurring is to scavenge initiator molecules also known as free radicals.[19] Thus, it was essential to identify the capabilities of *P. cypria* to scavenge a synthetic free radical. In the DPPH• assay, an artificial nitrogen based free radical interacts as both as a free radical and as an indicator compound. After reduction reactions DPPH• is reduced to the hydrazine form which is shown as a color change from purple to colorless at 517 nm.[19] In the literature, other *Phlomis* species tested using DPPH• radical and obtained different IC_{50} data (0.14-12.18 mg/mL) from the different extracts.[28] Our results found between these limits. Non-polar extracts, hexane and dichloromethane, did not show any statistically significant radical scavenging activities in this assay, potentially because of their solubility in polar media. $ABTS^{+\bullet}$, an artificial radical with a rather stable nitrogen core, is more versatile in use, as both polar and non-polar compounds

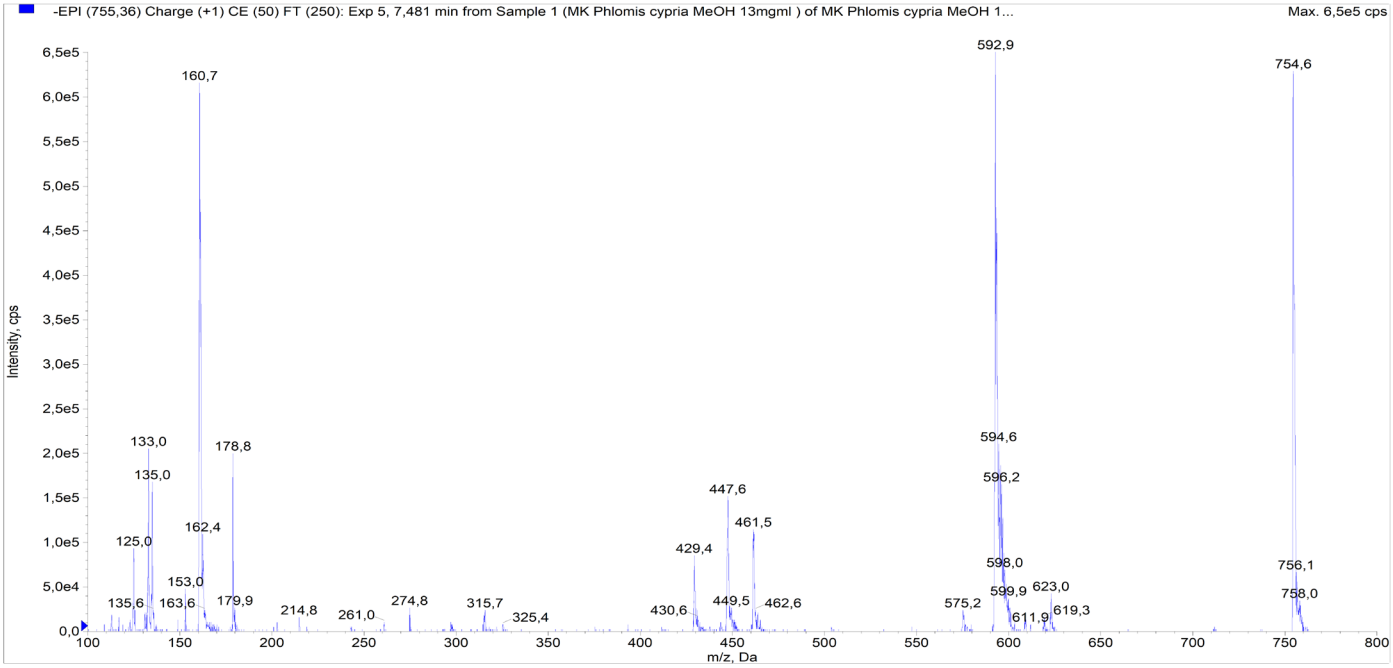

Figure 5: LC MS/MS spectrum of forsythoside B.

can be examined and the spectral inference can be kept to a minimum due to the maximum absorption wave used, which is not normally encountered by natural products (760 nm).[20] In principle, $ABTS^{+\bullet}$ is similar to $DPPH^{\bullet}$ but with the broader range of interaction due to its chemistry. Polar extract of *P. cypria* also found to be more active in $ABTS^{+\bullet}$ assay same as $DPPH^{\bullet}$. Phenolic compounds are known as responsible compounds within the extracts due to the antiradical activities.

The ability of chemicals to inhibit acetylcholine esterase is a field of interest. As acetylcholineesterase inhibitors are used in the treatment of nerve poisoning and neurodegenerative diseases such as Parkinson's and Alzheimer's disease. These compounds are able to inhibit acetylcholine esterase allowing for the accumulation of the neurotransmitter acetylcholine, which is necessary for cognitive and behavioral functions. In the literature, highest enzyme activities from the different *Phlomis* species were also obtained from the ethyl acetate extracts which contained the middle polarity compounds.[28]

CONCLUSION

The results of LC MS/MS analysis showed that forsythoside B was the main active compound within the polar extract. Caffeic acid derivatives were also found in the methanolic extract, which is most active extract of *P. cypria*. Plomis species have been investigated for different effects such as antibacterial, anti-inflammatory, anticancer, antidiabetic, antifungal and antiparasitic. . As a result of these studies, it was reported that essential oil fraction had antibacterial effect while polar fraction was responsible for other effects. All results from the biological activity and chemical composition assays within this study were the first records for *P. cypria*. Our future research will focus on the enrichment of the active extracts and illuminating the mechanisms of these biological activities.

ACKNOWLEDGEMENT

The authors acknowledge the efforts of Mr. Sami Thomson who collected the plant material from North Cyprus.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Author Contribution Statement

M. T. T., I.K. and M. K. conceived and designed and extracted the plant material and antiradical activities, F. G. analyzed the extract in LC MS/MS, N. Z. and İ. K. performed the cell culture assays, M. İ. related with antimicrobial assays, T. E. investigated the extracts in cholinesterase enzyme systems, M. T. T., N. Z., İ. K., T. E., M. İ., F. G. and M. K. wrote the article.

ABBREVIATIONS

SK-HEP1: Human Hepatic Adenocarcinoma; **DMEM:** Dulbecco's Modified Eagle's Medium; **FBS:** Fetal bovine serum; **NEAA:** Non-essential amino acids; **PBS:** Phosphate buffer solution; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor; **DCFH-DA:** 2'-7'dichlorofluorescein diacetate; **DCF:** 2',7'–dichlorofluorescein; **HEPES buffer:** 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer; **LC MS/MS:** Liquid Chromatography; **AChE:** Acetylcholine esterase; **BChE:** Buthyrylcholine esterase; **HPLC:** High Performance Liquid

Chromatography; **TEAC:** Trolox Equivalent Antioxidant Capacity; **DPPH•:** 1,1-Diphenyl-2-picrylhydrazyl; **ABTS$^{+•}$:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **DMSO:** Dimethyl sulfoxide; **DTNB:** 5,5'-Dithio-bis(2-nitrobenzoic) acid.

SUMMARY

Phlomis cypria Post (Lamiaceae), an endemic plant from North Cyprus, was extracted with hexane, dichloromethane and 70% aqueous methanol using maceration at room temperature. All extracts were tested for antiradical (DPPH• and ABTS$^{+•}$), anticholinesterase, antibacterial and the cytotoxic activities and their chemical compositions were analyzed by LC MS/MS method as well. The antiradical activities of aqueous methanol extract were found as IC_{50} 0.29 mg/mL in DPPH• and TEAC 1.22, 1.36 in ABTS$^{+•}$ assays. Dichloromethane extract only showed activity in anticholinesterase assay (inhibitions were 37.68% for AChE; 21.73% for BChE). Forsytoside B was found as the main phenolic compound in the active extract with caffeic acid bound to other compounds.

None of the extracts showed antibacterial activity and only aqueous methanol extract showed cytotoxic activity with 1264 µg/mL IC_{50} value.

REFERENCES

1. Venkateshappa SM, Sreenath KP. Potential medicinal plants of Lamiaceae. AIJRFANS. 2013;3:82-7.
2. Fedorov AA. Flora of Russia, the European part and bordering region. Vol. 3. Portland: Timber Press; 2000.
3. Khanam M, Hassan M. A critical study of the genus Leucas R. Br (Lamiaceae) from Bangladesh. Bangla JOL. 1970;12:1-10.
4. Singh KN, Lal B. Ethnomedicines used against four common ailments by the tribal communities of Lahaul-Spiti in western Himalaya. J Ethnopharmacol. 2008;115(1):147-59. doi: 10.1016/j.jep.2007.09.017, PMID 17980527.
5. Sarac N, Ugur A. Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla. Eurasian J Biosci. 2007;4:28-37.
6. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. Iran J Pharm Res. 2003;2:77-82.
7. Liu P, Takaishi Y, Duan HQ. Two new phenylethanoid glycosides from the roots of Phlomis umbrosa. Chin Chem Lett. 2007;18(2):155-7. doi: 10.1016/j.cclet.2006.12.019.
8. Law WSJ, Salick J. Comparing conservation priorities for useful plants among botanists and Tibetan doctors. Biodivers Conserv. 2007;16(6):1747-59. doi: 10.1007/s10531-006-9057-2.
9. Limem-Ben Amor I, Boubaker J, Ben Sgaier M, Skandrani I, Bhouri W, Neffati A *et al*. Phytochemistry and biological activities of Phlomis species. J Ethnopharmacol. 2009;125(2):183-202. doi: 10.1016/j.jep.2009.06.022, PMID 19563875.
10. Limem-Ben Amor I, Neffati A, Ben Sgaier M, Bhouri W, Boubaker J, Skandrani I *et al*. Antimicrobial activity of essential oils isolated from *Phlomis crinita* Cav. ssp. mauritanica Munby. J Am Oil Chem Soc. 2008;85(9):845-9. doi: 10.1007/s11746-008-1272-4.
11. Liolios C, Laouer H, Boulaacheb N, Gortzi O, Chinou I. Chemical composition and antimicrobial activity of the essential oil of Algerian *Phlomis bovei* De Noé subsp. bovei. Molecules. 2007;12(4):772-81. doi: 10.3390/12040772, PMID 17851429.
12. Zhang Y, Wang ZZ. Comparative analysis of essential oil components of three Phlomis species in Qinling Mountains of China. J Pharm Biomed Anal. 2008;47(1):213-7. doi: 10.1016/j.jpba.2007.12.027, PMID 18243625.
13. Morteza-Semnani K, Saeedi M. The essential oil composition of *Phlomis bruguieri* Desf. from Iran. Flavour Fragr J. 2005;20(3):344-6. doi: 10.1002/ffj.1459.
14. Aligiannis N, Kalpoutzakis E, Kyriakopoulou I, Mitaku S, Chinou IB. Essential oils of Phlomis species growing in Greece: Chemical composition and antimicrobial activity. Flavour Fragr J. 2004;19(4):320-4. doi: 10.1002/ffj.1305.
15. Kasai R, Katagiri M, Ohtani K, Yamasaki K, Chong-Ren Y, Tanaka O. Iridoid glycosides from Phlomis younghusbandii roots. Phytochemistry. 1994;36(4):967-70. doi: 10.1016/S0031-9422(00)90473-4.
16. Çalış I, Kırmızıbekmez H. Glycosides from Phlomis lunariifolia. Phytochemistry. 2004;65(18):2619-25. doi: 10.1016/j.phytochem.2004.04.038, PMID 15451327.
17. Flora of Cyprus [homepage on the Internet] [cited Aug 24, 2019]. Available from: http://www.flora-of-cyprus.eu/endemics.
18. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-78. doi: 10.1016/S0076-6879(99)99017-1.
19. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana. General Pharmacology: The Vascular System. 1999;32(6):661-7. doi: 10.1016/S0306-3623(98)00238-9.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radic Biol Med. 1998;26:1231-7.
21. Ellman GL, Courtney KD jr Andres V, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95. doi: 10.1016/0006-2952(61)90145-9, PMID 13726518.
22. Clifford MN, Johnston KL, Knight S, Kuhnert N. Hierarchical scheme for LC-MS n identification of chlorogenic acids. J Agric Food Chem. 2003;51(10):2900-11. doi: 10.1021/jf026187q, PMID 12720369.
23. Ersoz T, Harput US, Calis I, Donmez AA. Iridoid, phenylethanoid and monoterpene glycosides from Phlomis sieheana. Turk J Chem. 2002;26:1-8.
24. Aghakhani F, Kharazian N, Lori Gooini ZL. Flavonoid constituents of Phlomis (Lamiaceae) species using liquid chromatography mass spectrometry. Phytochem Anal. 2018;29(2):180-95. doi: 10.1002/pca.2733, PMID 28983983.
25. Hanoglu A. Near east university at Northern Cyprus [Ph.D. thesis]; 2019.
26. Marin PD, Veitch NC, Grayer RJ, Kite GC, Soković M, Janaćković P. Flavonoids from Phlomis fruticosa (Lamiaceae) growing in Montenegro. Biochem Syst Ecol. 2007;35(7):462-6. doi: 10.1016/j.bse.2007.01.001.
27. Ulubelen A, Topcu G, Kolak U. Studies in natural products chemistry ur Rahman A, editor. Elsevier; 2005.
28. Sarikurkcu C, Uren MC, Tepe B, Cengiz M, Kocak MS. Phenolic content, enzyme inhibitory and antioxidative activity potentials of *Phlomis nissolii* and *P. pungens* var. pungens. Ind Crops Prod. 2014;62:333-40. doi: 10.1016/j.indcrop.2014.09.002.

Cite this article: Kunter İ, Tarabishi MT, Zabib N, Erçetin T, İlktaç M, Göger F, *et al*. New Data for Endemic *Phlomis cypria* Post from North Cyprus: Biological Activities and LC MS/MS Analysis. Indian J of Pharmaceutical Education and Research. 2023;57(2):511-8.