Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (3):233-239
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Antioxidant activity of extracts, *cis* and *trans* tilirosides, and other compounds from *Thymelaea microphylla* Coss. et Dur.

Tarak Mekhelfi[1], Khawla Adouni[2], Lahcene Zaiter[1], Graziano Guella[3], Samir Benayache[1], Fadila Benayache[1] and Petras Rimantas Venskutonis[2*]

[1]*Unité de recherche Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques (Varenbiomol), Département de Chimie, Faculté des Sciences Exactes, Université Constantine 1, Constantine, Algérie*
[2]*Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas LT-50254, Lithuania*
[3]*Laboratorio di Chimica Bioorganica, Dipartimento di Fisica, Università di Trento, via Sommarive 14, I-38123 Povo, Trento, Italy*

ABSTRACT

In the present study, we report the following: isolation and structural elucidation of two previously unknown in Thymelaea microphylla compounds, ethyl caffeate ***1*** *and ladanein* ***2****; evaluation of antioxidant properties and total phenolic content (TPC) of T. microphylla extracts; photoisomerization of cis/trans-tilirosides and structure-antioxidant activity relationship of these two isomers. The petroleum ether and chloroform extracts were tested by DPPH*$^{\bullet}$*, ABTS* $^{\bullet+}$ *scavenging capacity (RSC) and ferric reducing antioxidant power (FRAP) assays. The results showed that there is a strong correlation between TPC and antioxidant activity of different T. microphylla extracts. Photoisomerization was induced by UV lamps at 365 nm, the percentage of cis-tiliroside was observed to increase up to 30%. Antioxidant activity was found to increase after phtoisomerization of tiliroside.*

Keywords*: T.* microphylla; flavone, ethyl caffeate; antioxidant activity; photoisomerization.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases due to their therapeutic benefits [1]. About 80% of the world populations rely on the use of traditional medicines, which are predominantly based on plant materials [2]. The scientific studies available on various medicinal plants indicate that promising phytochemical preparations can be developed for treating different human health problems [3]. The genus *Thymelaea* (Thymelaeaceae) consists of about 31 species of shrubs and herbs [4], 8 of them grow in Algeria [5]. Aerial parts of *T. hirsuta* are used for decoction in the treatment of diabetes [6]. *T. hirsuta* has also been used in folk medicine for its antimelano-genesis [7], antioxidant [8], hypoglycaemic and antidiabetic activities [9]. Phytochemical investigations of *T. microphylla* resulted in the isolation of various secondary metabolites such as daphnane, coumarins, flavonoids, lignans, sterols, acids and terpenes [10, 11, 12, 13]. However, the reports on *T. microphylla* antioxidant activity are rather scarce [14, 15, 16]. Herein we report the isolation and structural elucidation of two previously unknown in *T. microphylla* compounds; the relation between the antioxidant activity of the petroleum ether and chloroform extracts with total phenolic contents; photoisomerization of *cis/trans*-tilirosides and structure-antioxidant activity relationship of these two compounds.

MATERIALS AND METHODS

Plant material

Aerial parts of *T. microphylla* (Thymeleaceae) were collected at the flowering stage from M'Sila, (east of Algeria) and authenticated by Prof. N. Khalfallah (Frères Mentouri, Constantine 1 University) and Prof. M. Kaabache (University of Sétif 1). A voucher specimen has been deposited at the Herbarium of the VARENBIOMOL research unit, Frères Mentouri, Constantine 1 University. Leaves and flowers were air-dried and used for the maceration and extraction.

Extraction and isolation

Air-dried leaves and flowers (4980 g) of *T. microphylla* were macerated at room temperature in EtOH–H_2O (70:30, v/v) for 24 h, three times. The extract was filtered, concentrated and dissolved in H_2O (1950 mL) under magnetic agitation. The resulting solution was filtered and successively extracted with petroleum ether, $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo* at room temperature to obtain the following extracts: petroleum ether (6.4 g), chloroform (8.20 g), EtOAc (14.94 g) and *n*-butanol (56 g). A part of the chloroform extract (6.3 g) was fractionated by column chromatography (CC) on silica gel using increasing polarity solvents, petroleum ether, $CHCl_3$, acetone and MeOH to yield 26 fractions (F1–F26) obtained by combining the eluates on the basis of TLC analysis. Fraction F17 (162 mg, petroleum ether/$CHCl_3$ 10:90) was rechromatographed on a silica gel flash column using $CHCl_3$/ MeOH(9:1) as eluent and then with increasing percentages of MeOH to yield 6 sub-fractions. Sub-fraction 4 was subjected to preparative plates of silica gel $CHCl_3$/ MeOH (9.5:0.5) to give caffeic acid ethyl ester **1** (6.5 mg) [34]. Fraction F24 (233 mg, $CHCl_3$/acetone, 80:20) which was rechromatographed on a silica gel flash column using $CHCl_3$/MeOH (9:1) as eluent and then with increasing percentages of MeOH yielded 9 sub-fractions. Sub-fraction 6 was rechromatographed on a silica gel flash column using hexane/EtOAc (5:5) as eluent and then with increasing percentages of EtOAc to obtain 6 sub-fractions. Sub-fraction 4 gave after purification on preparative plates of silica gel eluted with $CHCl_3$ /MeOH (9:1) ladanein **2** (3 mg) [26]. *Trans*-tiliroside was isolated from the EtOAc extract [11].

Antioxidant assays

Determination of total phenolic content (TPC)

Ten µL of appropriate dilutions of extracts or gallic acid solutions were oxidized with 190 µL Folin-Ciocalteau's reagent solution in distilled water (1:13) [17]. The reagents were mixed, allowed to stand for 3 min and then neutralized with 100 µL of 7% Na_2CO_3. The mixture was vortexed for 90 min and the absorbance was read at 765 nm in a FLUOstar Omega reader. The TPC was calculated using gallic acid calibration curve and expressed in mg gallic acid equivalents per g of DW plant material (mg GAE/g).

$DPPH^{\bullet}$ scavenging capacity

Radical scavenging capacity (RSC) of plant extracts against stable-2,2-diphenyl-1- picrylhydrazyl hydrate ($DPPH^{\bullet}$, Sigma Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically by a slightly modified method of Brand-Williams and co- workers [18]. The solution of $DPPH^{\bullet}$ in MeOH (6.5×10^{-5}) M was prepared daily before measurements on a UV/visible light spectrophotometer Spectronic Genesys 8 (Thermo Spectronic, Rochester, NY) at 515 nm. Extract solutions were prepared by dissolving dry *T. microphylla* extract in MeOH. Two mL of $DPPH^{\bullet}$ solution were mixed with 50 µL of different concentrations (0.62, 1.25, 2.5 and 5 mg/mL) extract solution in 1 cm path length disposable microcuvette. The samples were kept in the dark for 30 min at room temperature and then the decrease in absorbance was measured. The absorbance of a blank sample containing the same amount of methanol and $DPPH^{\bullet}$ solution was prepared and measured daily. The experiment was carried out in triplicate. The RSC was calculated by the following formula: $I = [(A_B \times A_A)/A_B] \times 100$, where I is $DPPH^{\bullet}$ inhibition, %; A_B is the absorbance of a blank sample (t = 0 min); A_A is the absorbance of extract solution (t = 30 min). The amount of extract required to decrease the initial $DPPH^{\bullet}$ concentration in the reaction by 50% is referred as an effective concentration, IC_{50}.

$ABTS^{\bullet+}$ scavenging capacity

Trolox equivalent antioxidant capacity (TEAC) was measured by using $ABTS^{\bullet+}$ scavenging assay [19]. A stock solution of $ABTS^{\bullet+}$ (2 mM) was prepared by dissolving in 50 mL of phosphate buffered saline (PBS) obtained by dissolving 8.18 g NaCl, 0.27 g KH_2PO_4, 1.42 g Na_2HPO_4 and 0.15 g KCl in 1L of ultra pure water. If the pH was lower than 7.4, it was adjusted with NaOH. Ultra pure water was used to prepare 70 mM solution of $K_2S_2O_8$. $ABTS^{\bullet+}$ radical cation was produced by reacting 50 mL of $ABTS^{\bullet+}$ stock solution with 200 µL of $K_2S_2O_8$ solution and allowing the mixture to stand in the dark at room temperature for 15–16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the assessment of extracts, the $ABTS^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. Three milliliters of $ABTS^{\bullet+}$ solution were mixed with 30 µL MeOH solution of extract in 1 cm path length microcuvette. The absorbance was read at ambient temperature after 30 min. PBS solution was used as a blank sample. All

determinations were performed in triplicate. The percentage decrease of the absorbance at 734 nm was calculated by formula: I = $[(A_B \times A_A)/A_B] \times 100$, where I is $ABTS^{\bullet+}$ inhibition, %; A_B is the absorption of blank sample (t = 0 min); A_A is the absorption of extract solution (t = 30 min).

Ferric reducing antioxidant power (FRAP) assay

The ability of plant extracts to reduce ferric ion to the ferrous one (FRAP assay) is another indicator frequently used for assessing antioxidant power [20]. Ferrous ion (Fe^{2+}) produced in this assay forms a blue complex [Fe^{2+} -TPTZ] absorbing at 593 nm. Briefly, the stock solutions included 300 mM acetate buffer (0.467 g $C_2H_3NaO_2\times$ 3 H_2O and 250 mL H_2O), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3\times 6H_2O$ solution. The fresh working solution was prepared by mixing 100 mL acetate buffer, 10 mL TPTZ solution, 10 mL $FeCl_3\times 6H_2O$ solution and then warmed at 37^0C before using. Extract (50 µL) were allowed to react with 1.5 mL of the FRAP solution and 150 µL H_2O for 30 min in the dark condition. The change in the absorbance (D A 593 nm) between the final reading and A_0 was calculated for each sample and related to the D A 593 nm of a Fe^{2+} reference solution which was measured simultaneously.

RESULTS AND DISCUSSION

Structural identification

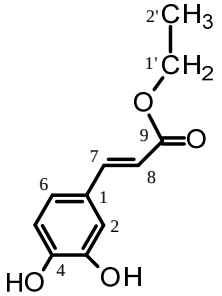
Description of the method

Nuclear magnetic resonance (NMR) spectra (^{1}H-NMR, ^{13}C-NMR, COSY, HSQC and HMBC) for all the isolated compounds were recorded in hexadeuterated dimethyl sulphoxide (99.9% CD_3SOCD_3), in deuterated chloroform (99.8%, $CDCl_3$) or in tetradeuterated methanol, (99.9%, CD_3OD) at 300 K on a Bruker-Avance 400 MHz NMR spectrometer by using a 5 mm BBI probe with 90^0 proton pulse length of 8.7 µs at a transmission power of 0 db. Liquid chromatography –mass spectrometry (LC/ESI-MS) measurements were performed on a Hewlett-Packard HP1100 HPLC-UV Diode Array system with an Esquire LC Bruker-Daltonics ion trap mass spectrometer using a reverse phase column Agilent Zorbax eclipse XDB-C18 (4.6 x150 mm x 3.5µm,with MeOH/H_2O and a flow rate of 0.9 mL/min using gradient elution. Mass spectra were obtained with an ESI source in positive-ion and negative-ion modes and analyzed by DataAnalysis (Version 3.0, Bruker Daltonik GmbH) and LC/MSD ChemStation (Agilent Technologies) software, respectively.

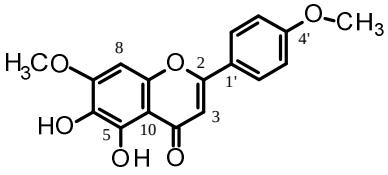
Isolated and identified compounds

Compound 1: brown powder; ^{1}H-NMR (400 MHz, acetone-d_6): δ (ppm) =7.52 (1H, d, *J* = 16 Hz, H-7), 7.12 (1H, brs, H-2), 6.99 (1H, brd, *J* = 7.6 Hz, H-6), 6.82 (1H, d, *J* = 7.6 Hz, H-5), 6.23 (1H, d, *J* =16 Hz, H-8), 4.17 (2H, q, *J* = 7.2 Hz, H_2-1'), 1.26 (3H, t, *J* = 7.2 Hz, H_3-2'); ^{13}C-NMR (100 MHz, acetone-d_6): δ (ppm) =166.23 (C, C-9), 148.45 (C, C-4), 145.40 (C, C-3), 144.70 (C, C-7), 126.70 (C, C-1), 121.39 (C, C-6), 115.38 (C, C-5), 114.15 (CH, C-8), 113.76 (CH, C-2), 59.63 (CH_2, C-1'), 13.95(CH_3, C-2'); (-) ESI-MS: *m/z* 179 $[M-H]^-$, according to the formula $C_{11}H_{12}O_4$ for this molecule. All these data led to ethyl caffeate [34]. This compound is reported in the genus *Thymelaea* for the first time.

Compound 2: Yellow powder, UV (MeOH, λ_{max}, nm): 276, 332; +NaOH: 276, 370 (with hypochromic effect); +$AlCl_3$: 294, 301sh, 355; +$AlCl_3$/HCl: 293, 351; +NaOAc: 276, 360, 364sh; + NaOAc/H_3BO_3: 276, 337; ^{1}H-NMR (400 MHz, acetone-d_6, δ (ppm): 8.02 (1H, d, *J* = 9.2 Hz, H2', H-6'), 7.11 (1H, d, *J* = 9.2 Hz, H-3', H-5'), 6.66 (1H, s, H-3), 6.64 (1H, s, H-8), 3.90 (3H, s, 7-OCH_3), 3.86 (3H, s, 4'-OCH_3). This compound which was identified as 5, 6-dihydroxy-7, 4'-dimethoxyflavone (ladanein) [26] was reported in the genus *Thymelaea* for the first time.



1



2

Total polyphenol contents (TPC)

The TPC values of the two plant extracts were determined using Folin-Ciocalteu reagent. Petroleum ether fraction had remarkably lower TPC (80.16±1.92 mg GAE/g) comparing to the chloroform fraction (151.55±8.06 mg GAE/g). This difference is easy to explain by the extraction procedure and solvents used: first step of extraction was

performed with polar solvent petroleum ether, the second one with medium polarity chloroform, whereas polyphenols are known to better dissolve in higher polarity solvents. Usually, the extracts containing high amounts of polyphenols also demonstrate high antioxidant activity.

DPPH$^{\bullet}$ scavenging capacity

The RSC of petroleum ether and chloroform extracts of *T. microphylla* extracts was analyzed using a DPPH$^{\bullet}$ participating in the single electron transfer reaction in the presence of a free radical scavenger; the absorption decreases and the resulting discoloration is stechiometrically related to the number of electrons gained [21]. The concentration dependent DPPH$^{\bullet}$ scavenging (%) activity is shown in Fig. 1. The chloroform extract inhibited 86.60% of radicals present in the reaction system, while petroleum ether extract demonstrated only 31.12% inhibition at 5 mg/mL; the IC_{50} values were 1.92 and 8.91 mg/mL, respectively. In the previous study, methanolic extract of *T. microphylla* showed an inhibition of 72% at remarkably lower concentration of 35 µL/mL [13] and also showed a significant and concentration-dependent free RSC (EC_{50}= 180.8 µg/mL) [12].

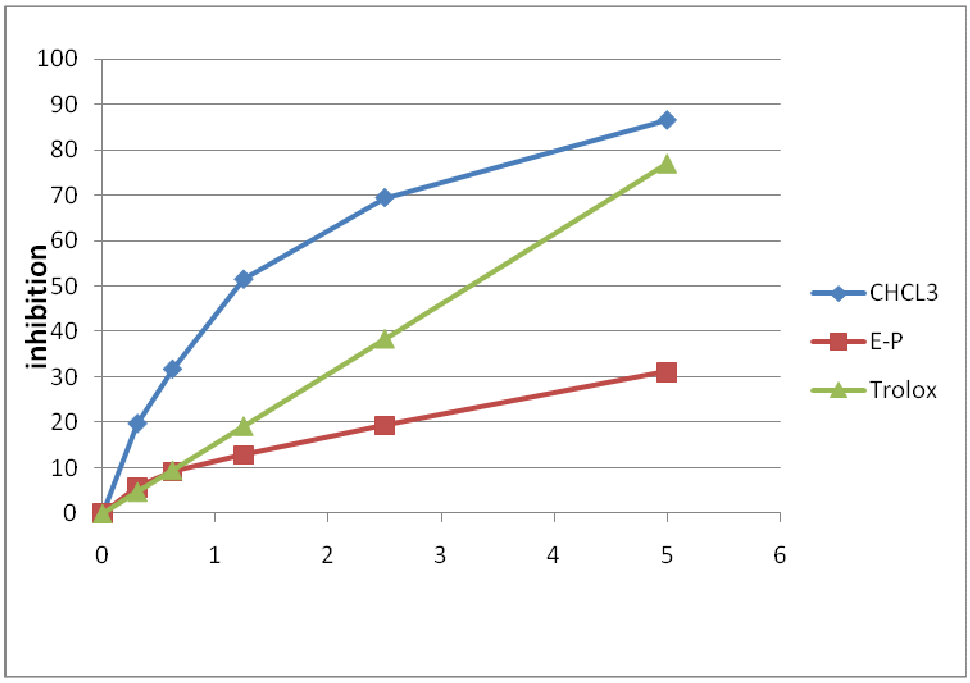


Fig. 1: DPPH$^{\bullet}$ Radical Scavenging Activity

ABTS$^{\bullet+}$ scavenging capacity

The ABTS$^{\bullet+}$ scavenging assay, which employs an absorbance at 734 nm wavelength and requires a short reaction time, can be used as an index that reflects the antioxidant activity of the test samples [22]. Chloroform and petroleum ether extracts were found to be effective in scavenging radicals and the increase was concentration-dependent (Fig. 2), the chloroform and petroleum ether extracts inhibited 88.63% and 18.09% of ABTS$^{\bullet+}$. At the concentration of 1.25 mg/mL; the IC_{50} values were 0.44 and 3.43 mg/mL, respectively. This show that *T. microphylla* extracts presents a good ability to scavenge the ABTS$^{\bullet+}$. The RSC of flower and stem extracts of *T. hirsuta* at 0.8 mg/mL concentration was 68.63 ± 0.53% and 43.04 ± 1.28) %, respectively, while leaf extract was weaker radical scavenger [23]. The antioxidant activities against ABTS$^{\bullet+}$ or DPPH$^{\bullet}$ were correlated with the concentration and chemical structures [23].

Table 1. DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging properties of chloroform ($CHCl_3$) and petroleum ether (PE) extracts of *Thymelaea microphylla* expressed as Trolox equivalent antioxidant capacity (TEAC, µmol TE/g) in extract (E) and plant material (PM) dry weight (DW)

Solvent	Extract yield, % (w/w)	TEAC (µmol/g) in ABTS$^{\bullet+}$		TEAC (µmol/g) in DPPH$^{\bullet}$	
		E	PM	E	PM
$CHCl_3$	0.16	1945±28	3.11	223.2±0.2	0.35
PE	0.120	214±2.7	0.25	133.2±0.4	0.15

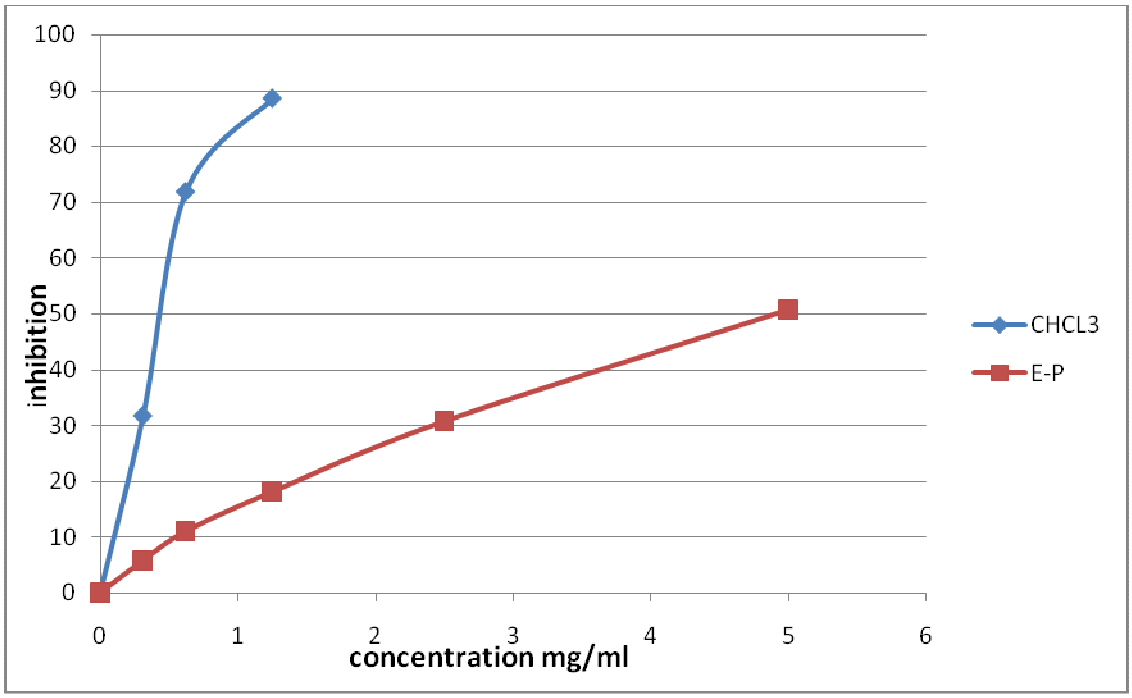


Fig. 2: ABTS^{+} scavenging capacity

FRAP assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyl triazine [Fe^{3+} -TPTZ] complex and producing a colored ferrous tripyridyl triazine [Fe^{2+} -TPTZ] [25]. FRAP assay evaluates the antioxidants in the sample as a reducing agents in a redox-linked colorimetric reaction [27]. In the present study, the trends for ferric ion reducing activities of petroleum ether and chloroform extracts are shown in Fig. 3. The absorbance of *T. microphylla* extract clearly increased, due to the formation of the [Fe^{2+} -TPTZ] complex with increasing concentration. The extracts showed increased ferric reducing power with the increased concentration [28]. Hence they should be able to donate electrons to free radicals stable in the actual biological and food system.

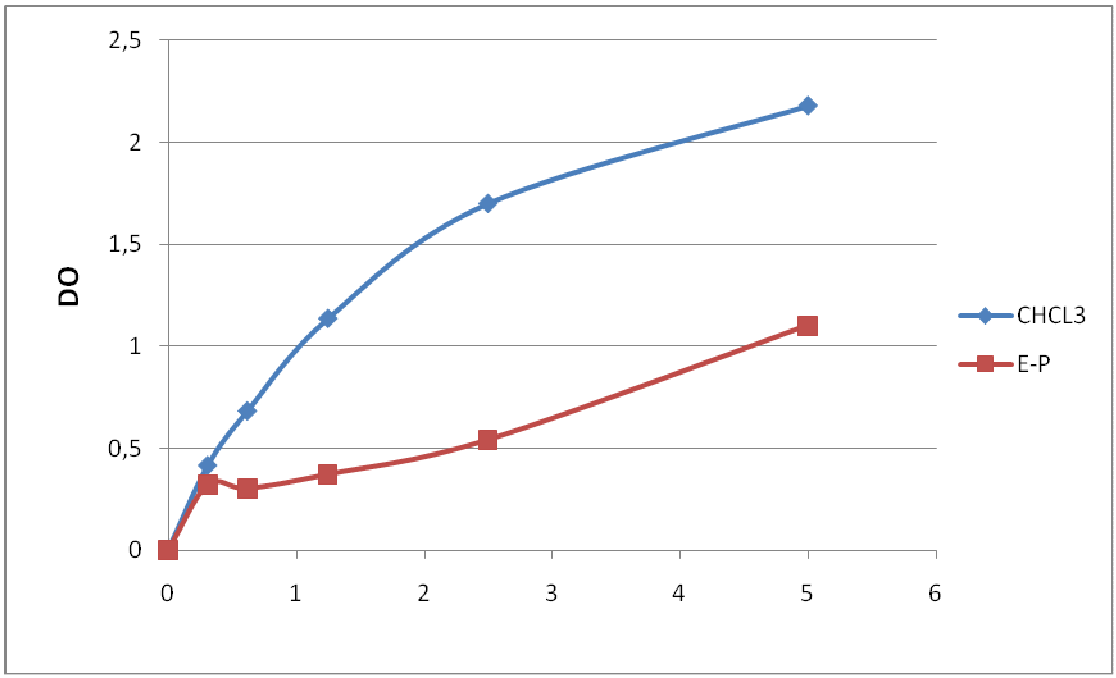


Fig. 3: FRAP assay

Table 2. Ferric reducing antioxidant power (FRAP) and total phenolic content (TPC) of chloroform ($CHCl_3$) and petroleum ether (PE) extracts of *Thymelaea microphylla*, expressed in Trolox equivalents (µmol TE/g) and gallic acid equivalents (TPC, mg GAE/g) respectively

Solvent	Yield, % (w/w)	TPC		TEAC $_{FRAP}$	
		E	PM	E	PM
$CHCl_3$	0.164	156.1±1.9	0.25	209.7±0.6	0.34
PE	0.128	80.2±1.9	0.10	223.1±3.4	0.28

Photoisomerization of tiliroside

Tiliroside was subject to photoisomerization with the use of UV (Applied photophysics SEMI MICRO photochemical reactor). The initial content of *cis*-tiliroside was 3% in the mixture of *cis* and *trans* isomers. The treatment of tiliroside by the light of 365 nm during 20 and 60 min increased the content of *cis*-tiliroside to 15% and 30%, respectively. Application of 254 nm light during 60 min was less effective; the content of *cis* isomer increased to 19%.

In-vitro antioxidant activity

Flavonoids and their glycosides have attracted considerable interest because of a large variety of biological activities, such as antioxidant [29], antiplasmodial [30], cytotoxic [31], anti-inflammatory [32] and antidiabetic [33]. However, no study has been reported on the effects of photoisomerization of flavonoids in relation to their biological activity. As it was reported in the previous section, UV photoisomerization allowed to obtain a mixture of *trans/cis*-tilirosides with a ratio of 70/30%, respectively. The antioxidant capacity of this mixture and pure *trans*-tiliroside was evaluated in DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP assays.

Table 3: percentage of inhibition (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) and FRAP (OD) of *trans*- tiliroside and *trans-cis* ti liroside (70%, 30%)

Concentration (mg/mL)	DPPH$^{\bullet}$ inhibition (%)		ABTS$^{\bullet+}$ inhibition (%)		FRAP (OD)	
	trans- tiliroside	mixture	*trans*- tiliroside	*trans*- tiliroside	mixture	*trans*- tiliroside
0.31	4.90	1.70	6.98	28.20	/	0.110
0.62	7.39	2.91	12.78	50.99	0.110	0.133
1.25	7.60	4.90	23.69	71.98	0.123	0.176
2.5	10.55	8.47	34.60	87.60	0.156	0.268
5	14.54	13.17	49.56	92.16	0.217	0.39
10	21.82	21.41	57.41	97.05	0.309	0.406

Table 3: The yields and Antioxidant characteristics of tiliroside: a pur flavonoid compound of *Thymelaea microphylla* and *trans /cis* photoisomerization. Values are expressed in µmol TE/g of extract.

Compounds	Yield, % (w/w)	ABTS$^{\bullet+}$	DPPH$^{\bullet}$	FRAP
tiliroside	24.39	152.6±2.2	36.5±0.69	28.37±1.02
***trans-cis* tiliroside (70%, 30%)**	0.24	261.9±4.7	38.32±2.07	18.46±0.49

The comparison of these results showed that no significant differences in DPPH$^{\bullet}$ values inhibition were found for *trans*-tiliroside and the mixture of tiliroside: the RSC of DPPH$^{\bullet}$ was almost similar for both products. However in ABTS$^{\bullet+}$ scavenged assay, the differences may be observed: the percentages of inhibition ranged from 6.98% to 57.41% for tiliroside and from 28.20% to 97.05% for the mixture of tilirosides. Thus the RSC of the mixture (*trans/cis*- tilirosides at 70/30%) in this assay was stronger antioxidant than pure *trans* isomer; the inhibition of ABTS$^{\bullet+}$ at 10 mg/mL was 57.41 % and 97.05%, respectively. The FRAP values of *trans*-tiliroside and the mixture of tilirosides steadily increased in by increasing the concentration of these two products; however in this reaction pure *trans*-tiliroside demonstrated higher antioxidant capacity than the mixture.

CONCLUSION

Chloroform extracts of *T. microphylla* demonstrated higher antioxidant potential comparing to unpolar petroleum ether extracts and it was evaluated by the content of phenolic compounds, radical scavenging capacity and reducing power. Consequently such extract may be a promising ingredient for antioxidant active preparation. Phenolic substances, which were previously reported in chloroform extract, may be the most important constituents responsible for the antioxidant activity of plant extracts. Photoisomerozation of *trans*-tiliroside in to the mixture of *trans/cis*-isomers (70/30%) resulted in the increase of antioxidant capacity in ABTS$^{\bullet+}$assay.

REFERENCES

[1] A. Nostro, M.P. Germanŏ, V. D'Angelo, A. Marino, M.A. Cannattelli, *Lett. Appl. Microbiol.*, **2000**, 30,379-384.

[2] Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines, (WHO. Regional Office for Western Pacific, Manila, **1993**) 2.
[3] D. Dahiru, E.T. William, M.S. Nadroi, *Afr. J. Biotechnol*., **2005**, 4(10), 1177-1179.
[4] D. Galicia-Herbada, *Pl. Syst. Evol*., **2006**, 257: 159–187.
[5] P. Quezel and S. Santa, Nouvelle flore de l'Algérie et des régions désertiques méridionales. Editions du C.N.R.S, Paris. **1963**, Tome II; 632-633.
[6] A. Ziyyat, A. Legssyer, H. Mekhfi, A. Dassouli, M. Serhrouchni, W. Benjelloun, J. *Ethnopharmacol*., **1997**, 58: 45–54.
[7] M. Kawano, K. Matsuyama, Y. Miyamae, H. Shinmoto, M. Elyes Kchouk, T. Morio, H. Shigemori, H. Isoda, *Exp. Dermatol*., **2007**,16,977–984.
[8] A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, *Food Chem*., **2006**,97, 654–660.
[9] F. El Amrani, A. Rhallab, T. Alaoui, K. El Badaoui, S. Chakir, *J. Med. Plants Res*., **2009**, 3 (9), 625–629.
[10] A. Cheriti, K. Sekkoum., *Acta Chim. Slovenica*, **1995**, 42(3), 373-374.
[11] T. Mekhelfi, K. Kerbab, G. Guella, L. Zaiter, S. Benayache, F. Benayache, *Der Pharmacia Lettre*., **2014**, 6 (1):152-156.
[12] K. Kerbab, T. Mekhelfi, L.Zaiter, S. Benayache, F. Benayache, P. Picerno, T. Mencherini, F. Sansone, R. P. Aquino, L. Rastrelli, *Natural Product Research*., **2014**, 7(29), 671-675.
[13] L. Said Noamane, A. Zellagui, K. Mesbah, N. Gherraf, M. Lahouel, S. Rhouati, *Der Pharmacia Lettre*., **2010**, 2(5), 428-431.
[14] A. Djeridane, M. Yousfi, J. M. Brunel, P. Stocker, *Food Chem Toxicol*., **2011**,48, 2599–2606.
[15] S. Ladjel, N. Gherraf, A. Zellagui, L. Brahim, S. Hameurlaine, *Plant Sci Feed*., **2011**, 1,179–182.
[16] L. Said Noamane, A. Zellagui, Y.Hallis, A. S. Yaglioglu, I. Demirtas, N. Gherraf, S. Rhouati, *Der Pharmacia Lettre*., **2015**, 7 (1):118-121.
[17] V. L. Singleton, R. Orthofer, R. M. Lamuela-Raventos, *Methods in Enzymology*., **1999**, 299, 152–178.
[18] W. Brand-Williams, M. Cuvelier, C. Berset, *LWT-Food Sci. Technol*., **1995**, 28, 25–30.
[19] R. N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans., *Free Radical Biology and Medicine*., **1999**,26, 1231–1237.
[20] F. F. Benzie, J. J. Strain, *Analytical Biochemistry*., **1996**, 239, 70–76.
[21] C.G. Silva, R.S. Herdeiro, C.J. Mathias, A.D. Panek, C.S. Silveira, V.P. Rodrigues, *Pharmacol Res*., **2005**, 52, 229-233.
[22] L.C. Wu, H.W. Hsu, Y.C. Chen, C.C. Chiu, Y.I. Lin, J.A. Ho, *Food Chem*., **2006**, 95, 319–327.
[23] N. Ouda Amari, M. ohamed Bouzouina, A. Berkani, B. Lotmani, *Asian Pac J Trop Dis*., **2014**, 4(2): 104-109.
[24] J. Oszmianski, A. Wojdylo, E. Lamer-Zarawska, K. Swiader, *Food Chem*., **2007**, 100, 579–583.
[25] I.F. Benzie, J.J. Strain, *Analytical Biochemistry*., **1996**, 239, 70–76.
[26] G. Gokdil, G. Topcu, U. Sonmez, A. Ulubelen, *Phytochemistry*., **1997**, 46, 799-800.
[27] C. Guo, J. Yang, J. Wei, Y. Li, J. Xu, Y. Jiang, *Nutrition Research*., **2003**, 23, 1719-1726.
[28] N. Benhammou, F. Atik Bekkara .J. Coustard, *Advances in Food Sciences*., **2009**,31, 194-201.
[29] J.T. Han, M.H. Bang, O.K. Chun, D.O. Kim, C.Y. Lee, N.I. Baek, *Arch Pharm Res*., **2004**, 27:390–5.
[30] A.W. Andayi, A. Yenesew, S. Derese, J.O. Midiwo, P.M. Gitu, O.J. Jondiko, *Planta Med*., **2006**, 72:187–9.
[31] Y.Y Xie, D. Yuan, J.Y. Yang, L.H. Wang, C.F. Wu, *J Asian Nat Prod Res*., **2009**, 11:771–8.
[32] D.D. Orhan, E. Küpeli, E. Yesilada, F. Ergun, *Z. Naturforsch*., **2006**, 61c, 26–30.
[33] Y. Zhu, Y. Zhang, Y. Liu, H. Chu, H. Duan,*Molecules*., **2010**, 15:9174–83.
[34] M. B. V. Saltos, B. F. N. Puente, N. Malafronte , A.Braca, *Journal of the Brazilian Chemical Society*.,**2014**, 25, 2121-2124.