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Anti-oxidative, acetylcholinesterase and pancreatic lipase inhibitory activities of compounds from *Dasiphora fruticosa, Myricaria alopecuroides* and *Sedum hybridum*

G.Odontuya

Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences, Peace Avenue, Ulaanbaatar 13330, Mongolia

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Abstract: Total of 35 phenolics and flavonols were isolated from flowers and leaves of *Dasiphora fruticosa* Rydb., from branches of *Myricaria alopecuroides* Schrenk. and from the herb of *Sedum hybridum* L. The isolated compounds were identified on the basis of spectral data and tested for their anti-oxidative, acetylcholinesterase and pancreatic lipase inhibitory activities.Quercetin glycosides, gallic acid, (-)EGCG and gossypetin-8-O-xylopyranoside tellimagrandin II exhibited strong anti-oxidative activity by the DPPH scavenging method. The acetylcholinesterase inhibitory activity of quercetin glycosides and (-)EGCG was higher than those of other compounds. Whereas, tellimagrandin II, (-)EGCG and gallic acid derivatives exhibited the most potent inhibitory activity against the pancreatic lipase enzyme among the isolated compounds. Only (-)EGCG showed a prominent activity against all assayed experiments. It was concluded that these plants could be studied further for their potential as anti-oxidative, anti-aging and lipid lowering active products.

Keywords: *Flavonol, gallic acid, tellimagrandin II, antioxidant, anti-aging, lipid lowering activity*

INTRODUCTION

Traditionally, flowers, leaves and scions of *Dasiphora fruticosa* Rydb. (Rosaceae) were used to cure diarrhea, dysentery and other digestive organs disorders, as well as for relieving the neurological disorders [1, 2]. Scions and branch of *Myricaria alopecuroides* Schrenk. (Tamaricaceae) have generally been used to neutralize the incidents of poisoning from different kinds of origins, to alleviate the diffused and concealed chronic fever and to treat several of diseases including rash, boils, chronic ulcers, phthisis, spasms and atrophy [1, 3, 4]. The herb of *Sedum hybridum* L. (Crassulaceae) has been used for the treatment of diarrhea, dysentery, sepsis, thyroid, blood vessel disease and seizures related with nervous system [1, 4, 5].

Previous studies revealed the presence of quercetin derivatives in the leaves of *D.fruticosa* [6, 7], gallic acid and its esterified derivatives in *M.alopecuroides* [8,9] and some flavonols and coumarins in *S.hybridum* [10].

The anti-oxidative, antibacterial activities of crude extracts, fractions and some compounds from the investigated plant samples have been partly reported [11-13]. As a part of continuing work on these plants, we report herein the content of bio-active compounds in the crude drugs, the characteristic phytochemicals and their anti-oxidative, acetylcholinesterase, and pancreatic lipase inhibitory activities.

*corresponding author: e-mail: g.odontuya@hotmail.com

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EXPERIMENTAL

General experimental procedures: Thin layer chromato chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} plate (Merck, Darmstadt, Germany) and the spots were detected under UV radiation (366 nm) by spraying with 1% methanolic diphenylboric acid-β-ethylamino ester (NP), 5% ethanolic polyethylene glycol (PEG). For column chromatography (CC) Sephadex LH-20 (25-100 mm, Pharmacia, Uppsala, Sweden), MCI gel – CHP-20P (75-150 μm, Mitsubishi Chemical Corporation, Japan), Sepra™ C18-E (50 μm, 65A°), Silica gel 60 (40-60 μm, Merck, Darmstadt, Germany) were used. UV spectroscopic analysis was carried out on spectrophotometer UV-160 (Shimadzu, Japan) using 5% $AlCl_3$, 1 N HCl, CH_3COONa and H_3BO_3 diagnostic reagents. All used reagents and solvents were with analytical grade. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on Bruker AMX-500, respectively. Quantitative analysis was carried out on spectrophotometer UV-2550 (Shimadzu, Japan).

Plant materials: All investigated plant samples were prepared from the Gobi-Altai aimag. Especially, flowers and leaves of *Dasiphora fruticosa* were collected from the mountain Khasagt Khairkhan, in June-July, 2004, flowered green branches of *Myricaria alopecuroides* from the riverside meadow, in late July, 2007 and aerial parts of *Sedum hybridum* from the ground mountain slope, in June-July, 2006. Prof. Jamsran Ts., Department of Botany, National University of Mongolia authenticated these plant species. Voucher specimens (Df 0406, Ma 0708, Sh 0607) have been deposited in the Herbariums of Natural Product

Chemistry Laboratory of ICCT, MAS.

Extraction and fractionation: Air dried and chopped crude drugs of *D.fruticosa, M.alopecuroides* and *S.hybridum* were extracted separately with 80% ethanol at room temperature exhaustively. Each total ethanol extract was filtered and concentrated under vacuum at 40°C to give the thick residue which was re-suspended in distilled water and further fractionated successively with equal volume of dichloromethane (DCM), ethylacetate (EA) and *n*-butanol (*n*-BuOH) (Table 1).

Table 1. Yield of extracts and fractions, g

Plant samples	Weight of drugs	Thick extract	DCM fraction	EA fraction,	n-BuOH fraction	Water residue
D.fruticosa (Df) flowers	300.0	93.0	5.1	25.8	17.3	40.0
D.fruticosa (Df) leaves	300.0	46.0	9.1	9.5	16.3	4.0
M.alopecuroides (Ma) branches	3500.0	818.0	68.4	40.0	250.0	200.0
S.hybridum (Sh) herb	1450.0	300.0	2.0	31.5	126.0	140.0

Each crude drug (50 g) was extracted twice with distilled water at room temperature and evaporated to dryness *in vacuo*. Then they were tested for their biological activities.

Isolation and identification: The EA fractions of Df flowers (25.5 g) and Sh herb (28.0 g), the EA and n-BuOH fractions of the Ma branch (35.0 and 30.0 g) were divided into several subfractions using CC with Sephadex LH 20, eluting with a mixture of 50% DCM in MeOH. Each subfraction was subjected to CC with different absorbents as 75-150 μm CHP 20P, and reversed phase sepra™ C18-E (50 μm) eluting with gradient H_2O – MeOH system, respectively. The final purification of substances was reached by repeated CC with Sephadex LH 20.

The DCM fractions of Ma branch (30.0 g), Df flowers (3.5 g) and Sh herb (1.0 g) were divided into numerous subfractions by CC with silica gel 60 (40-60 μm), eluting with *n*-hexane: DCM – 7:3→6:4→5:5→3:7→1:9 v/v, and DCM: EA – 10:1→95:5→9:1 v/v, respectively. The subfractions were separated repeatedly over silica gel columns eluted gradiently with solvent systems such as *n*-hexane : EA, $CHCl_3$: MeOH and DCM : MeOH.

From all three plant species compounds (see Fig. 2) **1** (0.34 g), **10** (0.42 g), **23** (0.39 g), **31** (0.50 g), **32** (0.17 g) **34** (0.44 g), from Df and Ma, compound **4** (0.45 g), from Df and Sh compound **6** (66.8 mg), from Ma and Sh compounds **24** (0.36 g) and **35** (0.31 g) have been isolated and identified. Only from Df compounds **2** (2.1 g), **3** (0.22 g), **5** (0.88 g), **11** (62.7 mg), **12** (0.48 g), **20** (0.20 g), **21** (0.34 mg) and **30** (8.0 mg), from Ma compounds **7** (7.6 mg), **8** (24.0 mg), **9** (24.0 mg), **13** (0.18 g), **14** (0.19 g), **17** (1.19 g), **27** (1.38 g) and **29** (0.28 g), from Sh compounds **15** (5.0 mg), **16** (26.2 mg), **18** (64.4 mg), **19** (33.7 mg), **22** (0.30 g) **25** (0.22 g), **26** (60.6 mg), **28** (22.3 mg) and **33** (67.8 mg) have been isolated and identified.

Quantitative analysis of total phenolics, flavonoids and tannins: The content of total phenolics in all crude drugs was measured by means of the Folin Ciocalteu reagent in pH<10, at 760 nm on a spectrophotometer. Results were expressed as gallic acid (Sigma Inc.) equivalent (GAE) from the calibration curve [14, 15]. The amount of total flavonoids was determined by spectrophotometry using a formation of acid stable complex with 2% $AlCl_3$ at 401.5 and 430.0 nm. Results were expressed as rutin (Sigma Inc.) and quercetin (TCI Co., Ltd) equivalents (RE and QE) from the calibration curve [16, 17]. The content of total tannins was determined by spectrophotometry using a hide powder and the Folin Ciocalteu reagent in pH<10, at 760 nm. Results were expressed as pyrogallol (Sigma Inc.) equivalent (PE) from the calibration curve [18].

DPPH scavenging activity: DPPH radical (2,2-diphenyl-2-picrylhydrazyl hydrate, TCI, Co., Ltd) scavenging activity of the crude extracts, fractions and pure compounds was determined according to the known spectrophotometric assay [19]. The absorbance was measured at 517 nm and the anti-oxidative activity (AA) was expressed in percentage:

$$AA\% = 100 - \{ [(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control} \};$$

Methanol (1.5 ml) added to plant extract solution (1.5 ml) was used as a blank. DPPH solution (1.5 ml, $6x10^{-5}$M) plus methanol (1.5 ml) was used as a control and rutin was used as a positive control.

Acetylcholinesterase inhibiting activity: The acetylcholinesterase (AChE) inhibiting activity of crude extracts, fractions and isolated pure substances was determined using a previously reported Ellman spectrophotometric method with DTNB (5,5'-dithiobis(2-nitrobenzoisc acid), TCI, Co., Ltd) color reagent [20]. The absorbance was measured at 412 nm and the AChE inhibiting activity (I) was expressed in percentage:

$$I (\%) = 100 \times (\Delta Abs_{control} - \Delta Abs_{sample})/\Delta Abs_{control}$$

$$\text{Where, } \Delta A_{control} = Abs_{positive\ control} - Abs_{blank\ positive\ control}$$

$$\Delta A_{sample} = Abs_{sample} - Abs_{blank\ sample}$$

Blank solution was prepared from 0.1 ml sample (1 mg/ml) with DTNB prepared in Tris-HCl, while the positive control was prepared from DMSO (dimethylsulfoxide) with the same amount of the sample. Eserin (physostigmine, TCI, Co., Ltd) was used as a positive control.

Pancreatic lipase inhibiting activity: The pancreatic lipase (PL) enzyme inhibiting activity of crude extracts, fractions and isolated pure substances was determined using a spectrophotometric method with DTNB color reagent [21]. The absorbance was measured at 412 nm and the PL inhibiting activity (I) was expressed in percentage:

$$I (\%) = (1-Abs_{sample}/Abs_{blank}) \times 100;$$

Blank solution was prepared from 0.1 ml of water with

DTNB prepared in Tris-HCl and porcine pancreatic lipase was prepared in water in the same amount as the sample analysis. Orlistat (TCI, Co., Ltd), a selective inhibitor for PL, was used as a positive control.

Statistical analysis: All data are expressed in mean ± standard deviation (S.D).

The IC_{50} value, defined as the amount of the sample that could reduce the initial concentration of DPPH, PL and AChE by 50%, was calculated from the linear regression plots of test samples concentration (μg/mL and mg/mL) against the mean inhibition in percentage. IC_{50} values were calculated using concentration of tested plant extracts, fractions and isolated substances and average percent of the antioxidant activity from three separate tests, PL and AChE inhibiting activities 6-8 separate tests.

RESULTS AND DISCUSSION

In general, the polar EA fractions of all investigated crude drugs of *D.fruticosa*, *M.alopecroides* and *S.hybridum* exhibited better anti-oxidative activity by the DPPH scavenging method and the inhibition activity against AChE and PL enzymes (Figure 1).

In particular, the IC_{50} value (mg/ml) of the EA fraction of *D.fruticosa* leaves for scavenging of DPPH was determined as 24.11±0.25, which is the greatest among the all studied samples, compared to the positive control rutin (22.66±0.29). The EA and *n*-BuOH fractions of the branch of *M.alopecuroides* (27.11±0.58 and 26.14±0.31) and the herb of *S.hybridum* (28.63±0.32 and 29.13±0.13) showed comparable results. The EtOH and water extracts, including the *n*-BuOH fraction of *D.fruticosa* samples gave almost twice lower activities than rutin. The ethanol extracts of *M.alopecuroides* and *S.hybridum* gave comparable results (31.93±0.48 and 35.61±0.36), which were better than the activity of related water extracts (52.35±0.85 and 48.59±0.64). The AChE inhibition percentage of all assayed samples at the 1 mg/ml concentration was considerably less than the positive control eserin, which can inhibit by 100% at the 0.1 mg/ml. However, in this tested concentration the EtOH extract, EA and *n*-BuOH fractions of *D.fruticosa* leaves, as well as the EA fraction of the branch of *M.alopecuroides* could inhibit by over 60%, while activities of other samples were in the 40-59% range. The PL inhibition percentages of all assayed samples were less than the positive control orlistat (62.5±1.27%) at the same 1 mg/ml concentration. The EA fractions of flowers and leaves of *D.fruticosa* gave the best activity as 50.25±0.86% and 48.82±1.27%, respectively, which were the closest results to orlistat. Whereas, the EA fractions of two other plant species and all EtOH extracts exhibited *ca.* 2-1.5 times lower activity than the positive control.

Nonpolar DCM fractions of all assayed samples did not contribute any effect to scavenging of DPPH, inhibiting of AChE and PL enzymes.

Results of biological activity experiments indicate that the EtOH extracts of all assayed plant samples exhibited better activity than the related water extracts and biologically active key components are presented in the polar EA and *n*-BuOH fractions.

Considering the results of biological activity assays and qualitative analysis, the total phenolics, flavonoids and tannins were determined by the spectrophotometric methods (Table 2). Total phenolics in flowers EtOH (17.05±0.96%) and water (14.80±1.41%) extracts of *D.fruticosa* were estimated in the highest amount among the ethanol and water extracts of all investigated samples. The amount of total flavonoids in the EtOH extract of *D.fruticosa* flowers was determined in rutin equivalent as 5.30±0.57%, which is more than twice higher than the EtOH extracts of two other plant species, while in quercetin equivalent as 5.89±0.11%, which is 3-9 times higher than the related extracts. The branch of *M.alopecuroides* was

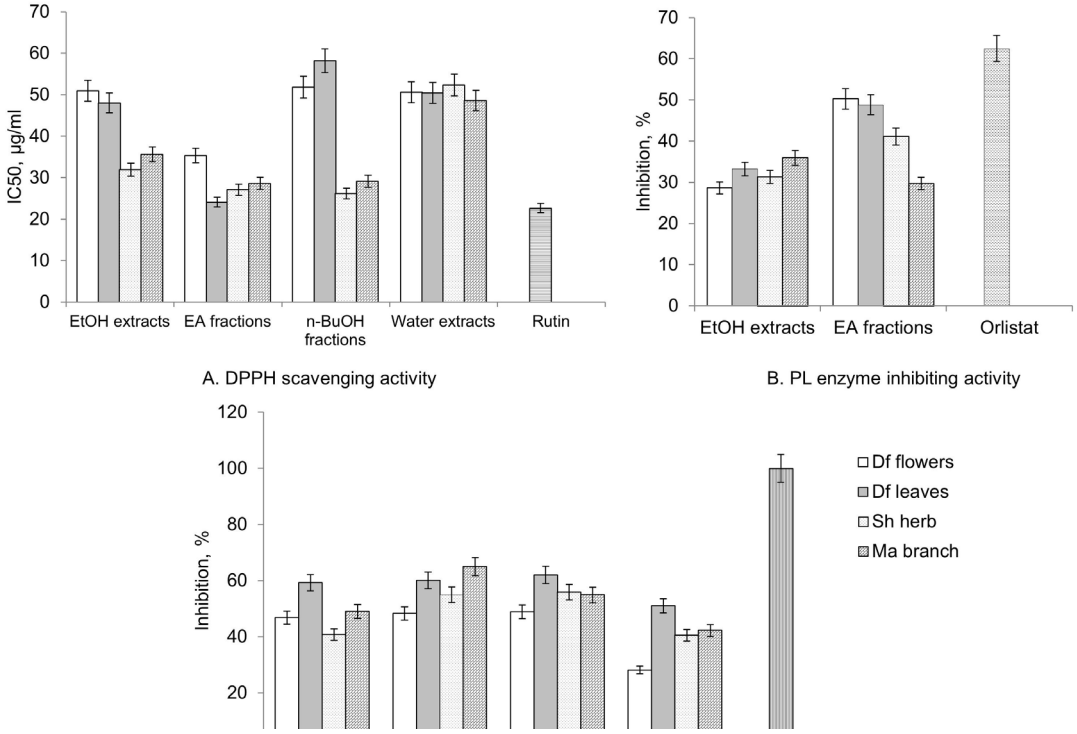


Fig. 1. Biological activities of crude extracts and fractions

Table 2. Quantity of main components, %

Samples			Total phenolics (in GAE)	Total flavonoids		Total tannins (in PE)
				(in RE)	(in QE)	
Dasiphora fruticosa Rydb.	Flowers	EtOH extract	17.05±0.96	5.30±0.57	5.89±0.11	4.88±0.15
		Water extract	14.80±1.41	5.17±0.16	2.90±0.09	
	Leaves	EtOH extract	12.16±0.74	4.51±0.22	4.36±0.13	3.40±0.07
		Water extract	8.45±1.92	2.42±0.08	2.76±0.01	
Myricaria alopecuroides Schrenk.	Branch	EtOH extract	15.14±1.48	2.09±0.03	0.61±0.02	5.33±0.11
Sedum hybridum L.	Herb	EtOH extract	9.44±0.62	1.98±0.07	1.54±0.03	3.32±0.05

specified by containing rather more amounts of tannins (5.33±0.11%), while its quantity of total phenolics was almost comparable to flowers and leaves of *D.fruticosa*. The EtOH extract of the herb of *S.hybridum* contains a bit lower amount of phenolics and tannins (9.44±0.62% and 3.32±0.05%) than other two plants related samples, while the amount of flavonoids was close to *M.alopecuroides*. Consequently, the qualitative and quantitative analysis of these three species indicated that they could classify to the phenolics containing plants.

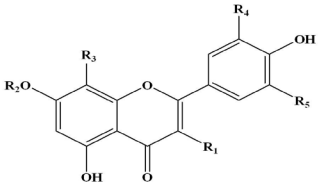
The biological activity results guide to isolate and identify altogether 35 known compounds from the flowers of *D.fruticosa*, from the branch of *M.alopecuroides* and from the herb of *S.hybridum* (Figure 2, Table 3). Among them, 29 phenolics, namely, quercetin-, kaempferol-, myricetin-aglycones and glycosides, gossypetin-, herbacetin- and apigenin-glycosides (**1-20**), (+)catechin (**21**), (-) epigallocatechingallate (EGCG) (**22**), gallic acid (**23**), ethylgallate (**24**), 6-O-galloylarbutin (**25**), 2,4,6-trigalloyl-O-*β*-*D*-glucopyranose (**26**), tellimagrandin II (**27**), caffeic acid (**28**) and ferulic acid (**29**) have been isolated from the EA and *n*-BuOH fractions of three plant samples. Whereas, other 6 compounds as fatty acids, ursolic acid and sterols (**30-35**) have been isolated from DCM fractions.

Quercetin glycosides (**2-6**) were evaluated as characteristic phytochemicals in flowers and leaves of *D.fruticosa*, while isorhamnetin (**17**), ethyl gallate (**24**) and tellimagrandin II (**27**) were specified the branch of *M.alopecoroides*. The herb of *S.hybridum* was differed from other two plants by containing of myricetin-, gossypetin-, herbacetin- aglycones and glycosides (**15-19**), and (-)EGCG (**22**). In the meantime, quercetin (**1**), kaempferol (**10**) and gallic acid (**23**) were found to be as common metabolites in these investigated plants. Another aim of this study was to evaluate the DPPH scavenging activity and the inhibitory effects against AChE and PL of isolated compounds (Table 3).

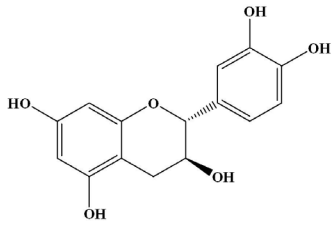
Generally, the anti-oxidative activity of flavonoids and phenolic compounds was studied very well by various methods [51]. According to the DPPH scavenging experiment 12 compounds, including quercetin glycosides (**2-7**), gossypetin-8-*β*-*D*-xylopyranoside (**19**), (+)catechin (**21**), (-)EGCG (**22**), gallic acid (**23**), 2,4,6-trigalloyl-O-*β*-*D*-glucopyranose (**26**) and tellimagrandin II (**27**) exhibited a strong activity, which was better than the positive control rutin (IC_{50}, 38.7μM). Especially, it was clearly notable that compounds **27** (IC_{50}, 5.97 μM), **7** (IC_{50}, 13.40μM), **6** (IC_{50}, 17.45 μM) and **26** (IC_{50}, 17.91 μM) showed by 6.5-2.2 times higher activity than rutin. Among the active flavonol derivatives quercetin glycosides (**2-7**) exhibited better activity than **19,** as well as **10**, **17**, **15, 16** and **18**. Molecular structures of all active compounds were distinguished by the presence of number of hydroxy groups, a 2,3-double bond, orthodiphenolic structure and gallate moieties. The structure-activity relationship for antioxidation and scavenging of radicals was well studied and documented [52-54].

Thirty four isolated compounds have been tested for their AChE inhibitory activity by the Ellman method [20]. None of the compounds exhibited activity against AChE like the potential drug physostigmine, which was used as the positive control. However, quercetin and its glycosides (**1-8**), kaempferol-, myricetin-, herbacetin-, gossypetin glycosides (**12,16,18,19**) and (-)EGCG (**22**) were found to be more active than others. On the other hand, they inhibited AChE at the concentration 0.5 mg/mL by over 50%, which was comparable with the results reported previously [55,56]. Gallic acid derivatives **26** and **27** exerted a weak inhibitory activity against AChE rather than their DPPH scavenging activity. Gallic acid (**23**) and ethylgallate (**24**) were not active against AChE (see Table 3). Hence, it is noticed that both flavonols and gallic acid derivatives showed the strong anti-oxidative activity. However, flavonolos have exhibited remarkable AChE inhibitory effect, whereas effect of gallic acid derivatives on AChE was minimal. To date 128 flavonoids were found to possess AChE inhibitory activity [56]. In this study flavonoid derivatives **3-6, 9, 12-14, 16-21** and an other class of compounds **24-35** have been investigated for their AChE inhibitory activity for the first time. The PL enzyme inhibition activity assay of all isolated compounds demonstrated that only **22** (IC_{50}, 0.1 mM) and **27** (IC_{50}, 0.051 mM), including compound **26** (IC_{50}, 0.116 mM) were active in the same dose of the positive control orlistat. Moreover, compounds **25** and **26** (IC_{50}, 0.236 mM and 0.267 mM) have demonstrated slightly more activity than the other components. Structures of these compounds were differed from the rest of components by the presence of multihydroxy groups and esterified galloyl moities, which are essential for the PL enzyme inhibition [57-59].

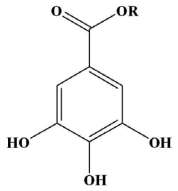
It is noteworthy that kaempferol glycosides (**11-14**) did not show any activity for scavenging of DPPH. Kaempferol and its glycosides (**10,11,13,14**), including **17**, **20**, **21**, **23**, **24** and **28** were also not active for the inhibition of AChE



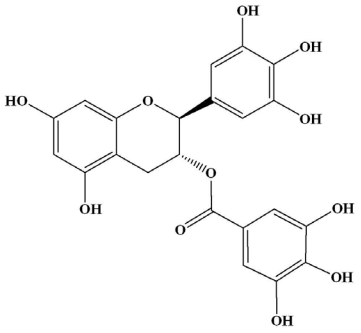
	R_1	R_2	R_3	R_4	R_5	Ref
1	OH	H	H	OH	H	22,23
2	O-α–*L*-arabinopyranoside	H	H	OH	H	22,24
3	O-(6'-galloyl)-*β-D*-galactopyranoside	H	H	OH	H	22
4	O-*β-D*-glucuronide	H	H	OH	H	25,26
5	O-*β-D*-galactopyranoside	H	H	OH	H	27
6	O-*L*-arabinofuranoside	H	H	OH	H	27
7	O-*β-D*-glucopyranoside	H	H	OH	H	28
8	O-α-*L*-rhamnopyranoside	H	H	OH	H	29
9	O-*β-D*-glucuronide methyl ester	H	H	OH	H	30
10	OH	H	H	H	H	27,28
11	O-*β-D*-glucopyranoside	H	H	H	H	31
12	O-(6'-coumaroyl)-*β-D*-glucopyranoside	H	H	H	H	32
13	O-α-*L*-rhamnopyranoside	H	H	H	H	29
14	O-*β-D*-glucuronide	H	H	H	H	33
15	OH	H	H	OH	OH	34
16	O-α-*L*-arabinofuranoside	H	H	OH	OH	35
17	OH	H	H	OCH_3	H	27,36
18	OH	H	O-*β-D*-xylopyranoside	H	H	37,38
19	OH	H	O-*β-D*-xylopyranoside	OH	H	38
20	H	O-(6'-coumaroyl)-*β-D*-glucopyranoside	H	H	H	39



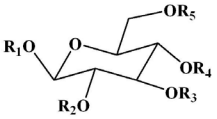
21 [40]



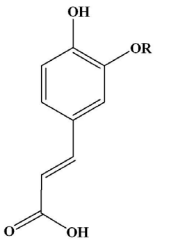
	R	Ref
23	H	42
24	CH_2CH_3	42
25	arbutoside	43



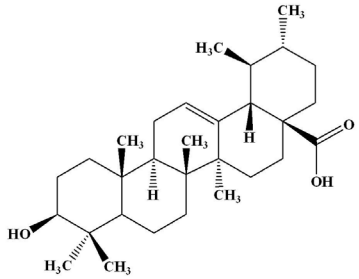
22 [41]



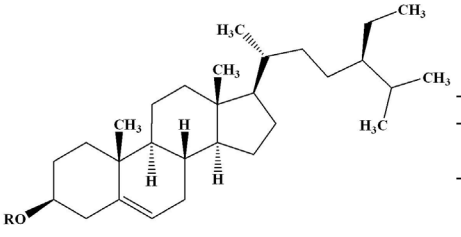
	R_1	R_2	R_3	R_4	R_5	Ref
26	H	galloyl	H	galloyl	galloyl	43
27	galloyl	galloyl	galloyl	6,6'-digalloyl		44



	R	Ref
28	H	45
29	CH_3	46



30 [47]



	R	Ref
31	H	48
32	*β-D*-glucopyranose	49

$C_{15}H_{31}COOH$

33 [50]

R_1-$COOR_2$

	R_1	R_2	Ref
34	$C_{17}H_{35}$	H	50
35	$C_{17}H_{35}$	CH_2CH_3	50

Fig. 2. Structures of isolated compounds

Table 3. Isolated compounds and their biological activities

Code	Compounds and references	Sources	DPPH scaven-ging activity IC_{50}, μM	AChE inhibiting activity, IC_{50}, mM	PL inhibiting activity, IC_{50}, mM
			Rutin IC_{50}, 38.7 μM	**Physostigmine IC_{50}, 0.000083 mM**	**Orlistat IC_{50}, 0.109 mM**
1	Quercetin	Df, Sh, Ma	41.36±0.89	0.098±0.001	1.784±0.030
2	Quercetin-3-O-α-*L*-arabinopyranoside	Df	**28.59**±0.11	0.021±0.003	1.865±0.027
3	Quercetin-3-O-(6”-galloyl)-*β*-*D*-galacto-pyranoside	Df	**31.68**±0.07	0.036±0.001	1.026±0.060
4	Quercetin-3-O-*β*--*D*-glucuronide	Df, Ma	**24.30**±0.27	0.077±0.002	1.191±0.018
5	Quercetin-3-O-*β*-*D*-galactopyranoside	Df	**25.77**±0.76	0.021±0.002	1.644±0.101
6	Quercetin-3-O-*α*-*L*-arabinofuranoside	Df, Sh	**17.45**±0.28	0.047±0.003	1.665±0.032
7	Quercetin-3-O-*β*-*D*-glucopyranoside	Ma	**13.40**±1.04	0.041±0.001	1.991±0.02?
8	Quercetin-3-O-*α*-*L*-rhamnopyranoside	Ma	ns	0.073±0.005	1.514±0.011
9	Quercetin-3-O-*β*--*D*-glucuronide methylat	Ma	ns	0.136±0.007	1.192±0.044
10	Kaempferol	Df, Sh, Ma	86.70±1.13	0.507±0.016	1.239±0.041
11	Kaempferol-3-O-*β*-*D*-glucopyranoside	Df	na	0.188±0.009	1.782±0.023
12	Kaempferol-3-O-*β*-*D*- (6”-*n*-coumaroyl)-glucopyranoside	Df	na	0.030±0.003	1.200±0.037
13	Kaempferol-3-O-*α*-*L*-rhamnopyranoside	Ma	na	0.349±0.011	1.804±0.072
14	Kaempferol-3-O-*β*−*D*- glucuronide	Ma	na	0.322±0.006	1.161±0.021
15	Myricetin	Sh	54.60±1.14	ns	ns
16	Myricetin-3-O-*α*-*L*-arabinofuranoside	Sh	41.95±0.13	0.081±0.001	1.267±0.027
17	Isorhamnetin	Ma	68.42±0.02	0.253±0.007	1.667±0.042
18	Herbacetin-8-O-*β*-*D*-xylopyranoside	Sh	50.17±1.17	0.051±0.001	1.116±0.011
19	Gossypetin-8-O-*β*-*D*-xylopyranoside	Sh	**35.18**±0.95	0.043±0.001	1.315±0.045
20	Apigenin-7-O-*β*-*D*-(6”-*n*-coumaroyl) gluco-pyranoside	Df	ns	0.325±0.007	1.076±0.094
21	(+) Catechin	Df	**36.21**±0.04	1.042±0.027	0.565±0.024
22	(-) Epigallocatechin-gallate	Sh	**19.63**±0.21	0.075±0.006	**0.100**±0.071
23	Gallic acid	Df, Sh, Ma	**30.90**±1.21	2.582±0.017	0.886±0.012
24	Ethylgallate	Sh, Ma	53.94±1.20	1.907±0.071	0.236±0.010
25	6-O-galloylarbutin	Sh	49.69±0.70	0.141±0.006	0.267±0.070
26	2,4,6-trigalloyl-O-*β*-*D*-glucopyranose	Sh	**17.91**±0.28	0.213±0.002	0.116±0.022
27	Tellimagrandin II	Ma	**5.97**±0.52	0.382±0.003	**0.051**±0.001
28	Caffeic acid	Sh	58.93±1.01	5.121±0.021	0.774±0.001
29	Ferulic acid	Ma	na	1.370±0.027	0.772±0.007
30	Ursolic acid	Df	na	0.306±0.003	1.647±0.022
31	*β*-Sitosterol	Df, Sh, Ma	na	0.670±0.007	3.329±0.025
32	*β*-Sitosterylglucopyra-nose	Df, Sh, Ma	na	0.202±0.011	1.864±0.071
33	Palmitic acid	Df, Sh	na	0.575±0.023	4.824±0.017
34	Stearic acid	Df, Sh, Ma	na	1.080±0.053	3.715±0.081
35	Stearic acid ethylate	Sh, Ma	na	1.580±0.017	2.483±0.071

Notice: Df – flower; Sh – herb; Ma – branch; na – not active; ns – not screened;

enzyme. All flavonoid derivatives (**1-20**) showed no activity against PL enzyme. Moreover, compounds **29-35** did not exhibit any activity against all tested biological activities.

Only (-)EGCG, well known green tea component, has been found to be the active component. (-)EGCG exhibited as a potential antioxidant by the scavenging of DPPH, an active inhibitor against AChE enzyme or the supporter for the brain cognition function and an excellent inhibitor for the PL enzyme or preventer against accumulation of lipids. Previously, Zhao *et al*. [60], Okello *et al*. [61] and Nakai *et al*. [62] reported about these three activities of (-)EGCG.

CONCLUSIONS

The flowers and leaves of *Dasiphora fruticosa*, the branch of *Myricaria alopecuroides* and the herb of *Sedum hybridum* were rich in phenolics, which were identified as the major bio-active characteristic compounds. Quercetin-, myricetin-, gossypetin glycosides, kaempferol-3-O-*β*-*D*-(6"-coumaroyl)glucopyranoside and (-)EGCG exhibited as active inhibitors against the AChE enzyme associated with the anti-oxidative activity. Whereas, multigalloylglucopyranose derivatives and (-)EGCG demonstrated to be as the active inhibitors against the PL enzyme associated with the anti-oxidative activity.

Consequently, the flowers of *Dasiphora fruticosa* and the herb of *Sedum hybridum* could be identified as antioxidants and AChE enzyme inhibitors, the branch of *Myricaria alopecuroides* as the antioxidant and the PL enzyme inhibitor. Therefore, the crude drugs of these plants could be studied further in view of their potential uses as anti-oxidative, brain anti-aging and lipid lowering active product

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