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

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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

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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.

EXPERIMENTAL

General experimental procedures: Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F 

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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), SepraTM C18-E (50 μm, 65A3), 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% AlCl3, 1 N HCl, CH3COONa and H2BO3 diagnostic reagents. All used reagents and solvents were with analytical grade. 'H (500 MHz) and 13C NMR (125 MHz) spectra were recorded on Brucker 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-Altaï 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. Jamshir 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).

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 sepraTM C18-E (50 μm) eluting with gradient H₂O – 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, CHCl₃: 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% AlCl₃ 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 was measured at 517 nm and the anti-oxidative activity (AA) was expressed in percentage:

\[
AA\% = 100 - \left\{ \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}} \right\}
\]

Methanol (1.5 ml) added to plant extract solution (1.5 ml) was used as a blank. DPPH solution (1.5 ml, 6x10⁻⁴ 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 Eillman spectrophotometric method with DTNB (5,5'-dithiobis(2-nitrobenzoic acid), TCI, Co., Ltd) color reagent [20]. The absorbance was measured at 412 nm and the AChE inhibiting activity (I) was expressed in percentage:

\[
I(\%) = 100 \times \frac{(\Delta\text{Abs}_{\text{control}} - \Delta\text{Abs}_{\text{sample}})}{\Delta\text{Abs}_{\text{control}}} \times \frac{\Delta\text{Abs}_{\text{sample}}}{\Delta\text{Abs}_{\text{blank}}}
\]

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 with a previously reported spectrophotometric method using DTNB (5,5'-dithiobis(2-nitrobenzoic acid), TCI, Co., Ltd) color reagent [21]. The absorbance was measured at 412 nm and the PL inhibiting activity (I) was expressed in percentage:

\[
I(\%) = \frac{(1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{blank}})}{\text{Abs}_{\text{blank}}} \times 100;
\]

Blank solution was prepared from 0.1 ml of water with

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**Table 1. Yield of extracts and fractions, g**

<table>
<thead>
<tr>
<th>Plant plants</th>
<th>Weight of drugs</th>
<th>Thick extract</th>
<th>DCM fraction</th>
<th>EA fraction,</th>
<th>n-BuOH fraction</th>
<th>Water residue</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. fruticosa</em></td>
<td>300.0</td>
<td>93.0</td>
<td>5.1</td>
<td>25.8</td>
<td>17.3</td>
<td>40.0</td>
</tr>
<tr>
<td><em>D. fruticosa</em></td>
<td>300.0</td>
<td>46.0</td>
<td>9.1</td>
<td>9.5</td>
<td>16.3</td>
<td>4.0</td>
</tr>
<tr>
<td><em>M. alopecuroides</em></td>
<td>3500.0</td>
<td>818.0</td>
<td>68.4</td>
<td>40.0</td>
<td>250.0</td>
<td>200.0</td>
</tr>
<tr>
<td><em>S. hybridum</em></td>
<td>1450.0</td>
<td>300.0</td>
<td>2.0</td>
<td>31.5</td>
<td>126.0</td>
<td>140.0</td>
</tr>
</tbody>
</table>
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.alopecruroides* 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.alopecruroides* (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.alopecruroides* 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.alopecruroides* 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 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.alopecruroides* was.
specified by containing rather more amounts of tannins (5.33±0.08%), while its quantity of total phenolics was almost comparable to flowers and leaves of *D. fruticosa*. The EtOH extract of the herb of *S. hybrida* 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. hybrida* (Figure 2, Table 3). Among them, 29 phenolics, namely, quercetin-, kaempferol-, myricetin- aglycones and glycosides, gossypetin-, herbacetin- and apigenin-glycosides (1-20), (+)-catechin (21), (-)-epigallocatechin (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. alopecuroides*. The herb of *S. hybrida* 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-6-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}, 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 phystostigmine, 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 moieties, 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.

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**Table 2. Quantity of main components, %**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolics (in GAE)</th>
<th>Total flavonoids (in RE)</th>
<th>Total tannins (in PE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dasiphora fruticosa</em> Rydb. Flowers</td>
<td>EtOH extract</td>
<td>17.05±0.96</td>
<td>5.30±0.57</td>
</tr>
<tr>
<td></td>
<td>Water extract</td>
<td>14.80±1.41</td>
<td>5.17±0.16</td>
</tr>
<tr>
<td>Leaves</td>
<td>EtOH extract</td>
<td>12.16±0.74</td>
<td>4.51±0.22</td>
</tr>
<tr>
<td></td>
<td>Water extract</td>
<td>8.45±1.92</td>
<td>2.42±0.08</td>
</tr>
<tr>
<td><em>Myricaria alopecuroides</em> Schrenk. Branch</td>
<td>EtOH extract</td>
<td>15.14±1.48</td>
<td>2.09±0.03</td>
</tr>
<tr>
<td><strong>Sedum hybridum</strong> L. Herb</td>
<td>EtOH extract</td>
<td>9.44±0.62</td>
<td>1.98±0.07</td>
</tr>
</tbody>
</table>
Fig. 2. Structures of isolated compounds
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, multigalloyl glucopyranoside 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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