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Anti-inflammatory, anticholinesterase and antioxidative constituents from the roots and the leaves of *Salvia nipponica* Miq. var. *formosana*

Hsiu-Hui Chan[a], Tsong-Long Hwang[b], Chung-Ren Su[a], Mopur Vijaya Bhaskar Reddy[a], Tian-Shung Wu[a,c,d,*]

[a] Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, ROC
[b] Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan, ROC
[c] Department of Pharmacy, China Medical University, Taichung 404, Taiwan, ROC
[d] Chinese Medicinal Research and Development Center, China Medical University and Hospital, Taichung 404, Taiwan, ROC

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ABSTRACT

Reactive oxygen species and granule proteases produced by neutrophils contribute to the pathogenesis of inflammatory diseases. The extracts of the roots and the leaves of *Salvia nipponica* var. *formosana* were showed potent inhibitory effects on superoxide anion production in fMLP/CB-activated human neutrophils as well as other anti-inflammatory effects, and led to the isolation of 25 compounds. Among them, compounds **8**, **12**, **13**, **14**, **15**, **17** and **20** were exhibited more potent inhibitory effect on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. Moreover, those isolated compounds also showed significant anticholinesterase and antioxidative activities. To the best of our knowledge, this is the first report of phytochemical and biological activity study on *S. nipponica* var. *formosana*.

1. Introduction

Salvia is an important genus consisting of about 900 species in the family Labiatae, many species which been used worldwide in folk-medicine from ancient times. The plants of this genus exhibited various biological and pharmacological activities, including antitumor, antioxidative, antimicrobial, antinociceptive, anti-angiogenic, anti-inflammatory and antiplatelet aggregation activities (Cardile et al., 2009; Jung et al., 2009; Ulubelen, 2003). Previously, we reported a new antitumor agent, neo-tanshinlactone, which was isolated from *S. miltiorrhiza* (Wang et al., 2004), and several notable new abietane diterpene alkaloids from *S. yunnanensis* (Lin et al., 2005). In our continuing search for biologically active compounds from *Salvia* species, we studied biologically activities and the constituents of the roots and the leaves of *Salvia nipponica* Miq. var. *formosana*, which grows at low elevation in Taiwan. To the best of our knowledge, there is no report on its phytochemical and pharmacological studies. In this investigation, we evaluated inhibitory effects of this plant on superoxide anion generation and elastase release by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). In addition, the anticholinesterase and antioxidative activities of the isolated compounds were also examined.

2. Materials and methods

2.1. Plant materials

The roots and leaves of *S. nipponica* var. *formosana* were collected from Taipei, Taiwan, in 2004. The plant material was identified by Prof. C.S. Kuoh (Department of Life Sciences, National Cheng Kung University). A voucher specimen (TSWu, 200409) has been deposited in the Department of Chemistry, National Cheng Kung University, Tainan, Taiwan.

2.2. Extraction and isolation

The roots of *S. nipponica* Miq. var. *formosana* (3.8 kg) were pulverized and extracted with methanol (6× 10 l) under reflux in 6 h. The filtrate was concentrated under reduced pressure to obtain crude methanol extract (340 g, SNR). This was suspended into H_2O, and successively partioned with $CHCl_3$ and *n*-BuOH to obtain $CHCl_3$ soluble syrup (15 g, SNRC), *n*-BuOH soluble portion (16 g, SNRB) and H_2O layer (305 g, SNRW). The leaves of *S. nipponica* Miq. var. *formosana* (4.8 kg), which were subjected the same as SNR, led to give SNL (540 g). SNL was suspended into H_2O, and successively partioned with $CHCl_3$ to obtain $CHCl_3$ soluble syrup (148 g). The

* Corresponding author at: Department of Chemistry, National Cheng Kung University, 1 Ta-Hsueh Road, Tainan 70101, Taiwan. Tel.: +886 6 2757575x65333; fax: +886 6 2740552.
E-mail address: tswu@mail.ncku.edu.tw (T.-S. Wu).

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$CHCl_3$ syrup was further partioned with *n*-hexane and 80% aqueous methanol to obtain *n*-hexane syrup (67 g, SNLH) and methanol syrup (72 g, SNLM). Compounds **1**–**25** were isolated from SNRC, SNRB, SNRW, SNLM and SNLH, respectively.

2.3. Preparation of human neutrophils and measurement of $O_2^{\bullet-}$ generation and elastase release

The methods of preparation of human neutrophils and measurement of $O_2^{\bullet-}$ generation and elastase release are described in our previously report (Lin et al., 2009).

2.4. Cholinesterase inhibitory assay

The inhibitory activities on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined by the modifying the procedures described in previous studies (Ellman et al., 1961; Kivrak et al., 2009).

2.5. Free radical scavenging activity assay

The DPPH• assay was described in our previously report (Chiu et al., 2005).

The isolation of all compounds and the pharmacological studies methods were described in detail in the supporting information.

3. Results and discussion

The phytochemical study of the methanolic extract of the roots and the leaves of *S. nipponica* var. *formosana* led to the isolation of 25 compounds, including taxodione (**1**), (+)-valeranone (**2**), nubiol (**3**), methyl rosemarinate (**4**), rosemarinic acid (**5**), 5-[(*E*)-2-carboxyvinyl]-2-(3,4-dihydroxyphenyl)-7-methoxy-2,3-dihydro-1-benzofuran-3-carboxylic acid (**6**), 3-(α-D-glucopyranosyloxy-methyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-(2*R*,3*S*)-dihydrobenzofuran (**7**), salvianolic acid B (**8**), a mixture of β-sitostrtol (**9**) and sigmasterol (**10**), β-sitosteroyl-3-*O*-β-D-glucoside (**11**), ursolic acid (**12**), 3-*epi*-corosolic acid (**13**), 2α,3α-23-trihydroxyurs-12-en-28-oic acid (**14**), oleanolic acid 3-*O*-ferulate(**15**), friedelin (**16**), caffeic acid methyl ester (**17**), octadecyl (*E*)-ferulate (**18**), *p*-coumaric acid methyl ester (**19**), vanillic acid (**20**), 3,4-dihydroxybenzaldehyde (**21**), tachioside (**22**), 13^2-hydroxy-(13^2-*S*)-phaeophytin a (**23**), adenosine (**24**), and nicotinic acid (**25**).

Neutrophil elastase is a major secreted product of stimulated neutrophils and a major contributor to the destruction of tissue in chronic inflammatory disease. Therefore, elastase is a target for therapy of chronic inflammatory diseases (Henriksen and Sallenave, 2008). As shown Fig. 1, SNL showed more potent inhibitory activity on superoxide anion and elastase release with

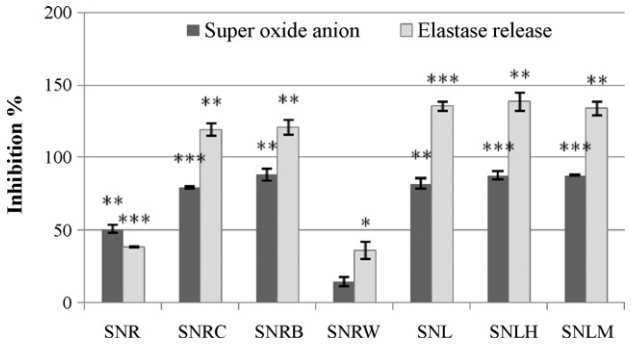

Fig. 1. Inhibitory effects of the extracts of *S. nipponica* var. *formosana* on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB.

Table 1
Inhibitory effects of compounds from *S. nipponica* Miq. var. *formosana* on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB.

No.	Superoxide anion IC_{50} (μM) or (Inh %)[a]	Elastase release IC_{50} (μM) or (Inh %)[a]
2	(23.86 ± 2.17)***	(36.68 ± 4.36)
3	(−9.16 ± 5.11)	(10.22 ± 2.98)**
4	N.T.	1.46 ± 0.42
5	N.T.	6.66 ± 0.87
8	26.00 ± 1.57	2.03 ± 0.37
11	(14.64 ± 4.23)*	(12.14 ± 0.82)
12	0.22 ± 0.02	0.33 ± 0.12
13	0.71 ± 0.05	1.29 ± 0.15
14	6.18 ± 0.19	6.00 ± 0.65
15	2.13 ± 0.44	1.23 ± 0.30*
17	0.44 ± 0.05	20.67 ± 3.00
20	11.76 ± 1.01	17.79 ± 2.24***
DPI[b]	1.02 ± 0.35	N.T.
PMSF[b]	N.T.	95.00 ± 25

[a] Percentage of inhibition (Inh %) at 30 μg/ml concentration. Results are presented as mean ± S.E.M. (n = 3–4). *p < 0.05, **p < 0.01 and ***p < 0.001 compared with the control value. N.T. means no test. Other compounds were not tested.

[b] Diphenyleneiodonium (DPI, a NADPH oxidase inhibitor) and phenylmethylsulfonyl fluoride (PMSF, a serine protease inhibitior) were used as the positive controls in the generation of superoxide anion and release of elastase, respectively.

the percentage of inhibition values of 82.33% and 135.6% at 10 μg/ml, while SNR exhibited 50.92% and 38.66% at same concentration, respectively. Furthermore, compounds **8**, **12**, **13**, **14**, **15**, **17** and **20** exhibited inhibition on fMLP/CB induced $O_2^{\bullet-}$ generation with IC_{50} values of 26.00, 0.22, 0.71, 6.18, 2.13, 0.44 and 11.76 μM, respectively (Table 1). Moreover, **4**, **5**, **8**, **12**, **13**, **14**, **15**, **17** and **20** showed more significant inhibitory effects on the release of neutrophil elastase than PMSF with an IC_{50} value of 95.00. Compound **12** is more potent than the corresponding hydroxyl substituted analog **13** in fMLP/CB induced inhibition of $O_2^{\bullet-}$ generation and elastase release in human neutrophils. The results clearly indicated triterpenoids, **12**, **13**, **14** and **15**, have all strong neutrophil pro-inflammatory activity while C-2 was unsubstituented. In present study, *S. nipponica* var. *formosana* contains triterpenoid compounds, such as **12**, **13**, **14** and **15**, which were major active constituents on inhibition of $O_2^{\bullet-}$ release and elastase release by human neutrophils.

Symptoms of Alzheimer's disease (AD) can be treated by the use of agents which restore the level of acetylcholine through inhibition of both the two major forms of cholinesterase (AChE and BChE). In the previously reports, the oils isolated from *Salvia* species exhibited inhibited activities for AChE or BChE (Kivrak et al., 2009; Loizzo et al., 2009). In our research, compounds **1**–**4** and **12** showed the inhibitory activities against BChE with IC_{50} values of 2.88, 114.12, 77.15, 108.51 and 46.70 μM, while no significant activities were found against AChE enzyme (IC_{50} >200 μM). Those isolated compounds could be considered as potent BChE inhibitors rather than AChE inhibitors.

The isolated compounds, except for **6**, **9**, **10**, **16**, **18** and **23**, were examined for their antioxidative properties using the α,α-diphenyl-β-picrylhydrazyl free radical (DPPH) scavenging assay. The results were compared with α-tocopherol, which was commonly used in the food industry as antioxidative (IC_{50}, 21.33 μM). Among them, compounds **4**, **5**, **17** and **20** showed strong DPPH radical-scavenging activity with IC_{50} values of 11.11, 11.89, 22.48 and 23.82 μM, respectively.

In summary, according bioactivities guided fractional process, this is the first report on the phytochemical study and biological activities of this species. The present study showed that this plant has a potential to develop an anti-inflammatory agent, an antioxidative agent or an anticholinesterase agent, particularly against BChE.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2010.06.017.

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