

# Isolation and Development of New Neuroprotective and Anticancer Agents from Medicinal Plants

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from Medicinal Plants

(薬用植物由来の新規神経保護物質および抗がん物質の探索)

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### 論 文 内 容 の 要 旨

Neurodiseases and cancer are the most common age-related diseases. Both diseases are associated with high levels of cell-inflammation and reactive oxygen species. Since medicinal plants provide an unlimited source for finding new medications, in this study, *Angelica shikokiana* (*A. shikokiana*); a Japanese medicinal plant was investigated for neuroprotection and cytotoxic activities. The Comparative biological study was conducted for ethanol and water extracts of different parts of the plant. The ethanol extracts of the stems and leaves showed the strongest anti-inflammatory and the highest antioxidant activities indicating their effectiveness in neurodiseases and cancer treatment. So, the aerial part of the *A. shikokiana* was used to isolate the neuroprotective and cytotoxic compounds.

Alzheimer's disease (AD) was taken as a model of neurodiseases. Pathologically, AD is associated with decrease of the neurotransmitter acetylcholine (ACh), high levels of oxidative stress and accumulation of amyloid beta fragments ( $A\beta$ ). So, bio-guided isolation of the neuroprotective compounds from the aerial part of *A. shikokiana* was conducted and resulted in the isolation of 22 compounds. *In vitro* neuroprotective assays showed that phenolic and coumarin compounds were the active neuroprotective principles of *A. shikokiana*. Quercetin, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside were the active agents against acetylcholine esterase enzyme (the key enzyme responsible for hydrolysis of the neurotransmitter; acetylcholine) through binding to its active site. Quercetin, luteolin, chlorogenic acid and methyl chlorogenate could protect against  $H_2O_2$  induced-neurotoxicity by scavenging of hydroxyl radicals and intracellular reactive oxygen species. Kaempferol-3-*O*-rutinoside and isoeopoxypteryxin were the active principles against  $A\beta_{25-35}$  induced-neurotoxicity through inhibition of amyloid fibril aggregation.

The Methanol extract of the aerial part (AME) and the isolated compounds were tested for their *in vitro* cytotoxic activities and selectivity using cancer and normal cell lines, respectively. The activities on tubulin polymerization and histone deacetylase 8 (as two targets of cytotoxicity) were examined to determine the mechanism of cytotoxicity. AME could inhibit tubulin polymerization and HDAC8 activity. Isolated compounds; Angelicin and kaempferol-3-*O*-rutinoside showed the strongest inhibition of tubulin polymerization through binding to colchicine binding domain of tubulin microtubules. Phenolic compounds; quercetin, luteolin, kaempferol, chlorogenic acid and methyl chlorogenate exhibited a strong inhibition of HDAC8 through binding to trichostatin A binding site.

*In vivo* hepatoprotective activities of AME and isoeopoxypteryxin (major compound; 13.29 mg/g w/w in alcoholic extract) were tested in rats against thioacetamide-induced hepatocellular carcinoma. Isoeopoxypteryxin showed for the first time potent *in vivo* hepatoprotective activities against TAA-induced HCC in rats. The hepatoprotective activity was correlated with several mechanisms including their ability to induce apoptosis by increasing the levels of caspase-3. Further, AME and isoeopoxypteryxin reduced

production of hepatic nitric oxide (NO) production by inhibition of inducible nitric oxide synthase (iNOS). Additionally, they inhibited the increased levels of vascular endothelial growth factor-C (VEGF-C) that are associated with HCC.

To develop more neuroprotective and cytotoxic active agents, the structure of isoeopoxypteryxin was changed by microbial biotransformation of isoeopoxypteryxin by the filamentous fungus *Cordyceps sinensis* to *cis*-khellactone (P1) and a new coumarin derivative, [(+)-*cis*-3'-[2''-methyl-3''-hydroxy-butanoyloxy]-4'-acetoxy-3', 4'-dihydroseselin (P2). P2 showed stronger cytotoxicity and higher selectivity against cancer cell lines by enhancing the inhibition of tubulin polymerization and histone deacetylase 8 (HDAC8). Similarly, P2 showed more neuroprotection against amyloid beta fragment 1-42 (A $\beta$ 1-42) induced neurotoxicity in human neuronal cells (SH-SY5Y) and exhibited more inhibition of the *in vitro* aggregation of A $\beta$ 1-42 than the parent compound.

To summarize, the aerial part of *A. shikokiana* was proved to have bifunctional activities; neuroprotection and cytotoxic activities. This study successfully found and could prepare new neuroprotective and cytotoxic compounds along with investigating their mechanism of the activity.