

Plants and their chemical compounds affecting β -amyloid and secretase activity as potential sources of neuroprotective herbal medicinal products. Part 2.

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Summary

In recent years, many herbal plants and their active components have been tested in different models of neurodegenerative diseases. Some studies are focused rather on studies of chemical compounds of plant origin than on plant extracts. Several natural polyphenols (i.e. flavonoids) are known to exhibit wide spectrum of beneficial effects on brain functioning and to protect against neurodegenerative processes [1, 2]. It seems that influence on β -amyloid is a promising point of pharmacological action of these plant components, because this protein is a major biological risk factor contributing to Alzheimer's disease (AD)-associated cascade including severe neuronal loss in the brain regions key for memory. In this review the attention is paid to studies on interesting natural chemical compounds of flavonoids (i.g. luteolin, myricetin, icariin) which are a promising study material for research of the potential neuroprotective effects by decreasing the activity of β -secretase (BACE-1) leading to diminish the generation and deposition of β -amyloid ($A\beta$) in the central nervous system. This range is even more interesting because plant polyphenols can be included in healthy diet and in multi-target drug therapy of neurodegenerative diseases.

Key words: *luteolin, myricetin, icariin, neuroprotection, β -amyloid, secretase*

1. LUTEOLIN

Chemical name and natural distribution

Luteolin is 5, 7, 3', 4' – tetrahydroxyflavone [3]. According to UPAC, this compound is named as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one [4].

Luteolin is one of the most common flavonoid present in different plants, especially as glycosides, for example as 8-C-glucoside (orientin), 6-C-glucosides (isoorientin), or 7-O-glucoside (cynaroside). Luteolin is widely distributed not only in many botanical families of Magnoliophyta, but also in Bryophyta, Pteridophyta and Pinophyta [5]. According to the review of Lopez-Lazata [5], this flavonoid and its glycosides occur in more than 213 species of plants, which are used as spice and also in traditional medicine. For example, luteolin has been found in *Apium graveolens*, *Capsicum annum*, *Capparis spinosa*, *Cucumis sativus*, *Cynara scolymus*, *Daucus carota*, *Fagopyrum esculentum*, *Mentha x piperita*, *Olea europaea*, *Origanum vulgare*, *Rosmarinus officinalis*, *Thymus vulgaris*. Moreover, luteolin as an aglycone and/or as glycosides may also occur in very popular medicinal plants affecting the central nervous system for example *Bacopa monieri*, *Ginkgo biloba*, *Hypericum perforatum* (and *H. brasiliense*), *Melissa officinalis*, *Passiflora incarnata* (and *P. edulis*, *P. caerulea*, *P. alata*), *Papaver rhoeas*, *Tanacetum parthenium*, *Vitex agnus-castus* [5], and *Centella asiatica* [6].

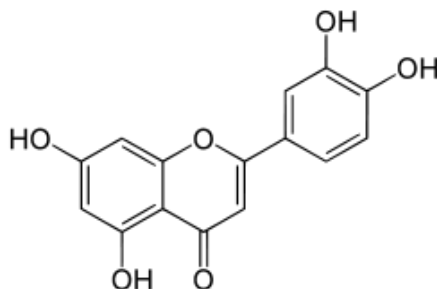


Figure 1.
Chemical structure of luteolin

Biological and pharmacological activities

Numerous studies suggest that luteolin has potential for the prevention and treatment of several diseases. This flavonoid exerts anti-inflammatory [5, 7], antimicrobial (antibacterial, antifungal) [5, 8], antioxidant [5, 9, 10] and anti-apoptotic activities [11, 12]. Moreover, it inhibits iodothyronine deiodinase, protein kinase C, NADH-oxidase, succinoxidase, lens aldose reductase [3]. Currently, an increasing number of studies suggest that luteolin has cancer preventive and therapeutic potential [5] for example in cell lines of the human breast cancer [13], human cervical carcinoma [14], human colon cancer [14, 15], lung cancer [16, 17] and prostate carcinoma [18].

Neuroactivity

In recent years, some data are available suggesting that luteolin and its glycosides interact with central nervous system cells (neurons and glial cells) and exert neuroprotective effects through different pathways. Results of *in vitro* study [19] showed that luteolin (isolated from the ripe seed of *Perilla frutescens*), significantly attenuated the increase in ROS production, markedly reversed hydrogen peroxide-induced cytotoxicity in primary culture cortical neurons and enhanced neuronal cell survival with efficacy higher than and potency similar to vitamin E. In other study [20] it was shown that luteolin protected rat neural PC12 and glial C6 cells from N-methyl-4-phenyl-pyridinium (MPP+) against toxicity induced *in vitro via* activation of the nuclear factor erythroid-2-related factor 2 (Nrf2), a transcription factor involved in the maintenance of the cellular redox homeostasis. Moreover, the neuroprotective effect of luteolin was observed in rat model of focal cerebral ischemia induced by permanent middle cerebral artery occlusion. It was shown

that luteolin (10 or 25 mg/kg, i.p.) administered after ischemia protected the brain against damage through oxidative stress and apoptosis reduction, and increased the activities of superoxide dismutase 1 (SOD1), CAT, Bcl-2 and claudin-5 [21]. It was also found out that the systemic administration of luteolin decreased neurologic impairment, and this effect may be through downregulation of toll-like receptors (TLR4, TLR5), nuclear factor- κ B (NF- κ B), mitogen-activated protein kinases (p38MAPK) and upregulation of extracellular signal-regulated kinase (ERK) expression [22]. Moreover, Qiao et al. [22] observed that luteolin reduced the neurologic deficit scores, brain edema and infarct volume after ischemia. They concluded that luteolin exerts its neuroprotective effect not only by antioxidant activity but also by anti-inflammatory property because luteolin affects the genes involved in immune responses. Similarly, the other studies demonstrated that luteolin decreased the release of pro-inflammatory cytokines in cultured microglia [23, 24]. Recent report has also confirmed that luteolin shows an antidepressant-like effect (50 mg/kg/d) in corticosterone-induced depression in mice model [25]. Moreover, it was demonstrated that luteolin (50, 150 and 450 mg/kg, p.o.) showed an enhancement of a basal synaptic transmission and facilitation of an induction of long-term potentiation (LTP) in the dentel gyrus of rat hippocampus in chronic cerebral hypoperfused rats [26]. This results allowed to state that luteolin not only can attenuate the cognitive deficits but also improve the synaptic plasticity in rats.

Anti-amyloid activity

Up to day, several studies were performed to investigate the impact of luteolin on gene expression and activity of β -secretase (BACE-1). More studies were focused on how luteolin affects the $A\beta$ -induced neurotoxicity. Choi et al. [27] showed that luteolin isolated from the methanolic extract of *Perilla frutescens* var. *acuta* exerted an inhibition of BACE-1 with IC50 values of 5.0×10^{-7} M. This activity was higher than result observed for rosmarinic acid (IC50 = 2.1×10^{-5} M). Other, very detailed studies [23, 28, 29] performed on SweAPP N2a cells and primary neuronal cells derived from transgenic Tg2576 mice allowed to state that luteolin exerted the antiamyloidogenic effects through inhibiting two forms of β -amyloid $A\beta$ 1-40,42 generation (with >70% and >85% reductions at treatment concentrations of 20 and 40 μ M, respectively) by reducing γ -secretase activity. Another study [30] demonstrated that luteolin isolated from *Elsholtzia rugulosa* exerted its neuroprotective effects on copper-induced neurotoxicity in the $A\beta$ precursor protein Swedish mutation stably overexpressing SH-SY5Y cells. Their positive results showed the decreasing expression of $A\beta$ precursor protein ($A\beta$ PP) and lowering of the secretion of $A\beta$ 1-42 observed after administration of luteolin. They also observed that luteolin increased cell viability and the activity of SOD and reduced

the release of reactive oxygen species (ROS). Cheng et al. [12] showed in cultured rat cortical neurons with $A\beta$ -induced toxicity that pretreatment with luteolin decreased apoptotic neuronal death by inhibiting the release of pro-inflammatory mediators. It is known that luteolin significantly inhibited the activation of the caspase-3 and could modulate mitogen-activated protein kinases which are key pathways in neuronal apoptosis [11]. Neuroprotective effect of luteolin on rat cerebral microvascular endothelial cells was also observed using *in vitro* model of $A\beta_{25-35}$ - induced toxicity [31]. *In vivo* studies performed in animals with $A\beta$ -induced cognition deficit showed that luteolin treatment improved the learning and memory processes both in rats (5, 10 mg/kg, p.o.) [32] and mice (5, 10 mg/kg, p.o.) [33]. It was also shown that luteolin increased the regional cerebral blood flow values and the brain-derived neurotrophic factor level [33]. Furthermore, it was discovered that this flavonoid administered in mice improved the cholinergic neuronal system activity through inhibition of acetylcholinesterase activity and increased an acetylcholine level in cerebral cortex [33]. Moreover, it was observed that luteolin also increased the level of SOD and glutathione (GSH) in cortex and hippocampus of rats [32].

2. MYRICETIN

Chemical name and natural distribution

Myricetin is a 3,5,7,3',4',5'-hexahydroxyflavone [3]. According to UPAC, this compound is named as 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)chromen-4-one [34].

Myricetin occurs free in the heartwood of *Soymidia febrifuga* (Meliaceae) and the aerial parts of *Haplopappus canescens* (Compositae). Glycosides of myricetin are widespread, e.g. the 3-glucoside occurs in the petals of *Primula sinensis* (Primulaceae), the 3-galactoside in the leaves of *Camellia sinensis* (Theaceae), and the 3-arabinoside in the berries of *Vaccinium macrocarpon* (Ericaceae) [3]. Myricetin was also found in stem bark of *Myrica esculenta* Buch. Ham. Ex D. Don, [35], and in bark of *Myrica rubra* Sieb. et Zucc. (Myricaceae) [36]. Myricetin was also detected in *Epilobium hirsutum* [9] and in extract of *Vitis vinifera* L. raisins [37]. In recent years phytochemical analysis carried out by Sultan and Anwar [38], showed that myricetin is contained in highest amounts in *Spinacia oleracea* (leaf) > *Brassica oleracea* (flower) > *Daucus carota* (root) > *Brassica rapa* (root) > *Pisum sativum* (seed). Moreover, myricetin occurs in fruits of *Prunus salicin* and *Fragaria ananassa*, and also the highest level of myricetin was detected in leaves of *Moringa oleifera* and of *Aloe barbadensis*, in fruits of *Ficus religiosa*, and in bark of *Acacia nilotica* [38].

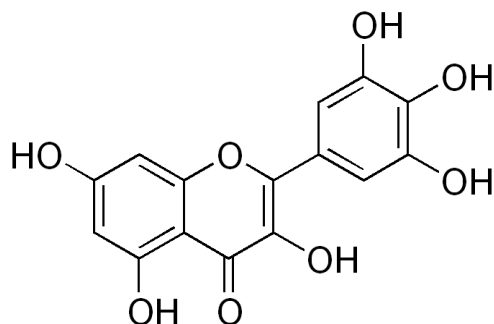


Figure 2.
Chemical structure of myricetin

Biological and pharmacological activities

Earlier studies showed that myricetin exhibited testosterone 5 α -reductase inhibitory activity and produced a significant anti-androgenic effect [36]. Other reports showed that myricetin possessed also a strong antigonadotropic properties and inhibited lipooxygenase, NADH-oxidase, succinoxidase activities [3]. Currently, myricetin is classified as a flavonoid which exerts strong antioxidant effects [39, 40].

Neuroactivity

Relatively new investigations allowed to state that myricetin showed neuroprotective properties *via* anti-amyloid and anti-secretase mechanism of action [39–41]. It was also shown that this compound produced neuroprotective effects in some Parkinson models through its antioxidant and antiapoptotic activity [42]. Moreover, myricetin exhibited a significant anxiolytic activity in behavioral models of anxiety by modifying serotonin transmission [37].

Anti-amyloid activity

Shimmyo et al. [39] used *in vitro* model of neurotoxicity to elucidate the effect of myricetin on secretion of β -amyloid and secretase activity. The cultured rat primary cortical neurons were treated with A β 1–42 (1 μ M) for 48 h. Obtained results allowed to state that myricetin in a concentration of 10 μ M significantly decreased the A β 1–40 and the A β 1–42 level in culture. Moreover, the aggregation of A β 1–42 to 65.2% was inhibited by myricetin at 1 μ M, and at 10 μ M. The results showed that myricetin

(10 μM , 48 h) reduced BACE1 activities of neuronal cells by 75% and IC_{50} was 2.8 μM . It was also shown that α -secretase activity in neuronal cells was increased by myricetin administration in a concentration-dependent manner. Another study of these authors [40] showed during the cell-free BACE-1 enzyme activity assay that not only myricetin but also quercetin, kaempferol, morin and apigenin directly inhibited BACE-1 enzymatic activity in a concentration dependent manner and values of IC_{50} were as follows: myricetin (2.8 μM) < quercetin (5.4 μM) < kaempferol (14.7 μM) < morin (21.7 μM) < apigenin (38.5 μM). Although, in the next step of this study it was shown that BACE-1 activity in neuronal cells was significantly decreased only after administration of myricetin and quercetin. Similarly, only these two flavonoids (after 24 h administration in neuronal culture at 20 μM) significantly decreased both $\text{A}\beta_{1-40}$ and $\text{A}\beta_{1-42}$ levels. Further study carried out by Wang and Jiji [41] with use of various spectroscopic methods such as circular dichroism (CD) and thioflavin T (ThT) fluorescence assay confirmed that myricetin inhibited $\text{A}\beta$ formation of hydrophobic fragment, $\text{A}\beta_{25-40}$, as well as $\text{A}\beta_{1-42}$. Moreover, CD and ultraviolet resonance Raman (UVR) studies indicated that myricetin induced the conformational change in both $\text{A}\beta_{25-40}$ and $\text{A}\beta_{1-42}$, suggesting that hydrophobic or backbone interactions may contribute to $\text{A}\beta$ -flavonoid binding. It was stated that myricetin produced interaction with soluble $\text{A}\beta$ via two mechanisms, association with hydrophobic C-terminal region and interactions with the aromatic side chains. Results of another study [43] with using molecular docking analysis allowed to state that myricetin interacts with the aspartate dyad by hydrogen bonding thus displacing the water molecule located between the aspartic acid pairs of Asp32 and Asp228 of BACE-1.

3. ICARIIN

Chemical name and natural distribution

Icariin ($\text{C}_{33}\text{H}_{40}\text{O}_{15}$) is classified to a flavonoids (main class) and also to a flavonol (sub class) According to UPAC, this compound is named as 5-hydroxy-2-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)-7-[(2S,4S,5S)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3-[(2S,3S,5R)-3,4,5-trihydroxy-6-methylloxan-2-yl]oxochromen-4-one [44].

The icariin is the major pharmacological active flavonoid which occurs in aerial parts of different plants from *Epimedium* species, for example of *Epimedium brevicornum* Maxim. [45], *E. sagittatum* Maxim. [46], *E. pubescens* Maxim. and *E. wushanense* T.S.Ying [47]. The largest number of species of *Epimedium* is found in China [47] but some are distributed in Japan, Europe, North Africa, India and Korea [49]. Nowadays, more than 141 flavonoids from 17 *Epimedium* species which belong to the family *Berberidaceae* [49] have been found.

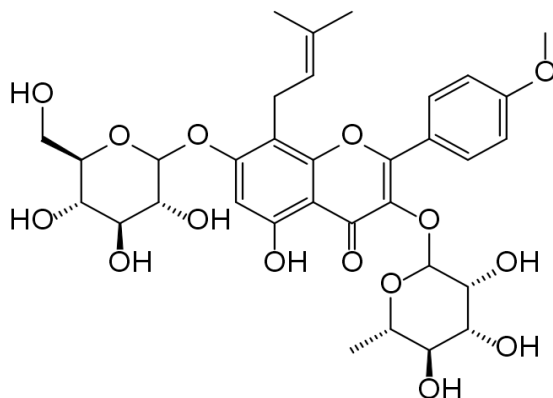


Figure 3.
Chemical structure of icariin

Biological and pharmacological activities

Several studies showed that bio-compounds of *Epimedium herba* increased the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and showed activity as free radicals scavengers. Moreover, icariin exerted protective activity against radical induced damage to DNA and peroxidation of polyunsaturated fatty acids [50]. It was also shown that flavonoids of *Epimedium* had protective effect against acute myocardial ischemia in rats [51]. According to recent review [49] modern preclinical pharmacological studies and clinical trials demonstrated that *Epimedium* extracts and their active compounds showed not only anti-oxidative properties but also possess wide pharmacological actions i.e. hormone regulation, anti-osteoporosis, immunological function modulation, as well as anti-tumor, anti-aging, anti-atherosclerosis, anti-hypertensive and anti-depressant activities. Moreover, in traditional Chinese medicine, icariin is used widely for the treatment of neurological diseases [52].

Neuroactivity

In recent years a neuroprotective effect of icariin in different pharmacological models [46, 52-59] was shown. Wang et al. [53] showed that this flavonoid produced neuroprotective effect against toxicity induced with A β 25-35 in primary cultured rat cortical neuronal cells (from embryonic rat fetuses). It was observed that pretreatment with icaritin (0.1 μ M) significantly reduced A-25-35-induced cell death by 90%, although, co-treatment with icaritin showed lower potency in this area. Moreover, co-treatment with estrogen receptor antagonist significantly

blocked neuroprotective effects of icariin. Authors suggested that neuroprotective effect of icariin was coupled with binding to estrogen receptor and, therefore, this antagonistic-like action is the potential mechanism of its neuroprotective activity. In another study, Zeng et al. [58] investigated the inhibitory effect of icariin on PC12 cells with $A\beta_{25-35}$ -induced tau protein hyperphosphorylation, which is one of the most representative hallmarks in AD. Results of this study showed that icariin application significantly decreased $A\beta_{25-35}$ -induced cytotoxicity and apoptosis rate through inhibiting tau protein hyperphosphorylation. Sha et al. [55] showed also that icariin (160 $\mu\text{g/ml}$) inhibited $A\beta_{42}$ -induced neurotoxicity *in vitro*. Moreover, Zeng et al. [58] concluded that icariin may activate PI3K/Akt signaling pathway, resulting in an inhibitory effect on glycogen synthase kinase (GSK)- 3β which is an important kinase included in tau protein hyperphosphorylation in pathogenesis of AD. Liu et al. [46] demonstrated also the neuroprotective effects of icariin against corticosterone-induced apoptosis examined in primary cultured rat hippocampal neuronal cells. It was observed that pre-treatment of neuronal cells with icariin suppressed corticosterone-induced cytotoxicity in a dose-dependent manner and western blot analysis showed that icariin blocked p38 MAPK phosphorylation. Moreover, Urano and Tohda [59] showed that icariin recovered $A\beta_{1-42}$ -induced neurite atrophy, when the icariin (0.01 μM) was administered 3 days after treatment with $A\beta$.

Anti-amyloid activity

Several studies have shown an interesting anti-amyloid activity [54, 56, 59]. In one of these studies, icariin was administered at the dosages of 30, 60 or 120 mg/kg for 14 days to Wistar rats which were unilaterally injected with $A\beta_{25-35}$ (10 μg) into right hippocampus [56]. Results of Morris water maze test indicated that flavonoid in the dosages of 60 and 120 mg/kg significantly improved the spatial learning and memory ability. Moreover, the real-time RT-PCR analysis showed that after the treatment with icariin the overexpression of BACE1 in rat's hippocampus induced by $A\beta_{25-35}$ was significantly decreased [56]. Neuroprotective effect of icariin was also found in another animal model. Urano and Tohda [59] demonstrated that administration of icariin (50 mmol/kg) for 8 days (p.o.) improved spatial memory impairment in transgenic mouse AD model (5xFAD - overexpression neuronspecific transgenes with five mutations). Moreover, it was shown in the Morris water maze that icariin significantly reduced the escape latency in 5xFAD mice, but object recognition memory and object location memory were sufficiently enhanced by icariin (50 $\mu\text{mol/kg}$) only in the normal mice. According to authors, these novel findings suggest that icariin may improve memory dysfunction in AD and have a potential to extend $A\beta$ -induced neurite atrophy. In another study, Luo et al. [54] examined the protective effects of icariin (60 and 120 mg/kg, by gavage for 3 months) against learning and memory deficits in aluminium-treated rats.

Their results showed that icariin dose-dependently protected against the development of aluminium-induced spatial learning and memory deficits (Morris water maze). Moreover, it was shown that icariin significantly increased SOD activity and decreased malondialdehyde (MDA) and A β 1-40 content in the hippocampus of aluminium-treated rats. These studies allowed to state that icariin may be promising option for the prevention of neurodegenerative processes.

CONCLUSION

In summary, this review showed that luteolin, myricetin and icariin occurring in many well-known plants, exerted their neuroprotective effects in different both *in vitro* and *in vivo* models. The most amounts of studies in neurodegeneration models were performed for luteolin, which is a component of spices, vegetables and medicinal plants. Taken together, luteolin exerts wide spectrum of neurobiological activities in models with using beta-amyloid as neurotoxin. Neuroprotective properties of luteolin may occur by increasing neuronal cell viability because this flavonoid inhibits the β -secretase (BACE-1) activity and may lead to down-regulation the β -amyloid precursor protein (A β PP) expression and diminish the secretion of A β 1-42. What more, luteolin may also inhibit acetylcholinesterase activity and improve the impairment of learning and memory performance induced by A β . Mechanism of action of luteolin may involve the decrease neuronal apoptosis by inhibiting the release of pro-inflammatory mediators and by reducing the release of reactive oxygen species. Therefore, luteolin is considered as a molecule to possible prevention of neurodegenerative diseases as multiple sclerosis [60], Alzheimer's disease, Parkinson disease, cerebral ischemia as well as for improving brain functions during aging [19]. It was also presented that in addition to strong antioxidant activity, myricetin may influence on amyloidogenesis. It was observed that this flavonoid inhibited aggregation of A β 1-42 and reduced BACE1 activity in neuronal cells. On the other hand, also icariin showed neuroprotective effect, not only by decreasing A β -induced cytotoxicity and apoptosis, but also by improving the learning and memory deficit in animal models for Alzheimer's disease. This effect might be caused by the inhibitory effect of icariin on BACE1 gene expression. According to several authors, these flavonoids will be great potential preventive and therapeutic agents for neurodegenerative processes in ageing brain. The more it becomes interesting because of the opinion that a diet enriched in flavonoids may influence the incidence and onset not only of cardiovascular diseases but also of neurodegenerative disorders, and plant-derived flavonoids may improve age-related impairment of memory and learning [2, 61-63]. However, it should be stressed that there is a need to perform further pharmacological and especially clinical studies in this area.

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ROŚLINY I ICH ZWIĄZKI CHEMICZNE WPŁYWAJĄCE NA B-AMYLOID I AKTYWNOŚĆ SEKRETAZ JAK O POTENCJA LNE ŹRÓDŁA NEUROPROTEKCYJNYCH PRODUKTÓW ZIOŁOWYCH. CZĘŚĆ 1.

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Streszczenie

W ostatnich latach liczne rośliny lecznicze, a także ich związki czynne bada się w różnych modelach chorób neurodegeneracyjnych. Coraz częściej badania koncentrują się bardziej na ocenie związków chemicznych pochodzenia roślinnego niż na badaniu ekstraktów roślinnych. Poznano kilka naturalnych związków polifenolowych (m.in. flawonoidy), które wykazują szerokie spektrum korzystnego wpływu na funkcjonowanie mózgu oraz w aspekcie aktywności cytoprotekcyjnej, zapobiegającej procesom neurodegeneracyjnym. Wydaje się, że obiecującym punktem uchwytu działania farmakologicznego tych związków roślinnych jest wpływ na β -amyloid, gdyż białko to jest ważnym czynnikiem ryzyka, istotnie przyczyniającym się do zachodzenia kaskady procesów prowadzących do choroby Alzheimera, a w tym do utraty neuronów w najważniejszych dla pamięci regionach mózgu. W artykule przeglądowym zwraca się uwagę na badania poświęcone interesującym związkom chemicznym z grupy flawonoidów (np. luteolina, myricetyna, ikaryna), które są obiecującym materiałem badawczym nad potencjalnymi działaniami neuroprotekcyjnymi poprzez obniżanie aktywności β -sekreazy (BACE-1), co może doprowadzić do zmniejszenia powstawania i odkładania β -amyloidu ($A\beta$) w ośrodkowym układzie nerwowym. Zakres tych badań jest jeszcze bardziej interesujący, gdyż polifenole roślinne mogą być zawarte w zdrowej diecie oraz mogą być włączane do złożonej terapii chorób neurodegeneracyjnych.

Słowa kluczowe: *luteolina, myricetyna, ikaryna, neuroprotekcja, β -amyloid, sekretaza*