

## NEW FINDINGS OF DOCTORAL DISSERTATION

Name of Doctoral candidate: **Nguyen Thi Thanh Loan**

Dissertation title: **Evaluating the pharmacological effects of persimmon leaves (*Diospyros kaki* L.f.) in the prevention and treatment of cerebral ischemia in experimental models**

Specialty: **Pharmacology - Clinical pharmacy**

Code of specialty: **9720205**

Name of academic advisors:

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2. Assoc. Prof. Dr. **Pham Thi Van Anh**

Name of academic Institute: **National Institute of Medicinal Materials**

### **Summary of new findings of the dissertation:**

#### ***1. Experimental models***

The study has successfully implemented both the *in vitro* and *in vivo* transient brain ischemic models to investigate the pharmacological effects of persimmon leaves (*Diospyros kaki* L.f.) in the prevention and treatment of cerebral ischemia:

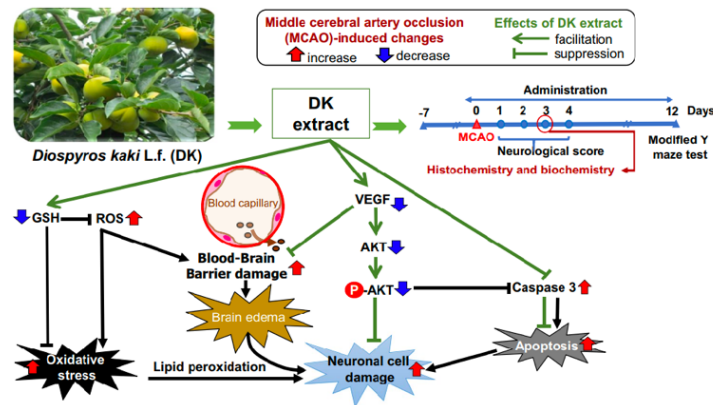
- Middle cerebral artery occlusion-and-reperfusion (MCAO/R) model and Organotypic hippocampal slice cultures (OHSCs) subjected to oxygen- and glucose-deprivation (OGD) were successfully performed as *in vitro* and *in vivo* transient brain ischemic models in Vietnam. In the present study, a mouse model of MCAO/R was successfully induced with cerebral infarction volume estimated to be 40% of the brain area using 2,3,5-triphenyl-tetrazolium chloride (TTC) staining; neurological symptoms were scored according to Menzies et al. In addition, mice were subjected to the modified Y-maze test to assess spatial working memory, a form of short-term memory. Based on the findings of the *in vitro* experiments, 60 minutes was the most suitable period for OGD to screen for the neuroprotective effects of pharmacological agents. Interestingly, organotypic hippocampal cultures retain a complex three-dimensional organization of nervous tissues, which are similar to that of the *in vivo* brain tissues. Because of the importance of the *in vivo*-like organization of nervous tissues of OHSCs, it is commonly used as an *in vitro* model for studying neuroprotective agents in neurodegenerative diseases.

- Furthermore, this study has proposed additional useful tools to determine other indicators of the cerebral infarction model, including evaluating the blood-brain barrier integrity using Evans blue dye extravasation, and determining the number of neuronal cells in certain brain regions through Nissl staining.

## 2. The pharmacological effects of a standardized flavonoid extract from *Diospyros kaki* L.f leaves (DK) in the prevention and treatment of cerebral ischemia in experiments

In this thesis, for the first time, the pharmacological effects of persimmon leaves in the prevention and treatment of cerebral ischemia were systematically studied. Thus, these features of DK may offer a beneficial therapeutic strategy for the ischemic insult:

- The results indicated the neuroprotective and therapeutic effects of DK on transient focal cerebral ischemic injury. Additionally, they provided underlying mechanisms using a MCAO/R model in mice, which is the most important outcome of this study. DK extract could exert protective and therapeutic effects on cerebral ischemia-induced brain damage by exhibiting anti-oxidative, anti-apoptotic, and pro-survival properties while preserving the blood–brain barrier integrity by acting through the VEGF/Akt signalling pathway. The results provide meaningful scientific evidence for future clinical trials evaluating DK as a potential treatment of ischemic strokes.



**Figure 1.** Neuroprotective effects of a standardized-flavonoid *Diospyros kaki* L.f. leaf extract (DK)

- Besides, this study demonstrated the effects of DK on some risk factors related to cerebral infarction, including hypertension and dyslipidemia. In part via angiotensin-converting enzyme activity inhibition, DK exerted the anti-hypertensive effects and alleviated ventricular hypertrophy. In addition, DK could control dyslipidemia through both

endogenous and exogenous mechanisms, contributing greatly to preventing cerebral infarction.

### 3. Neuroprotective effects against transient ischemia-induced brain damage of the potent flavonoid components isolated from the leaves of persimmon

The present results indicated that compound (9) (kaempferol-3-O-(2''-O-galloyl-β-D-glucopyranoside)) exhibited the potent neuroprotective activity against cerebral ischemic injury, suggesting the critical role of the flavonoid in the action of DK. Compound (9) at doses of 10 and 20 mg/kg exerted neuroprotective effects, at least in part, via the preservation of blood-brain barrier integrity and suppression of oxidative stress caused by ischemic insult. This paved the way for further research evaluating the neuroprotective effects of compound (9). Additionally, compound (9) can be identified as a biomarker in persimmon leaf extract for the standardization of leaves of persimmon.

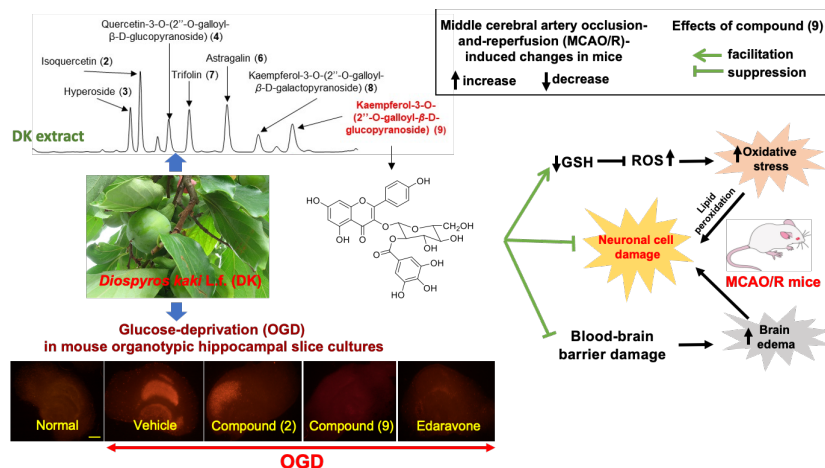


Figure 2. Neuroprotective effects of compound (9)

Hanoi, 30 November 2023

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