

# 東北医科薬科大学

## 審査学位論文（博士）要旨

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## Studies on three species of Mongolian medicinal plants

Phytochemical investigations and anti-parasitic activities of *Oxytropis lanata* and *Bergenia crassifolia*, and the effects of *Astragalus mongholicus* on uterus

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Last couple of decades, numerous research studies have been in focus on evaluation of biological activities and phytochemical constitutions of plants, especially medicinal plants, due to considered as a valuable and effective candidate for medical, cosmetic, and other field. The number of publications has revealed many investigations on various plant species; however there are still have many not investigated species and biological activities, which hopefully may be a source of valuable bioactive ingredients.

Herein, focused on to explore some biological activities and phytochemical ingredients of Mongolian medicinal plants, *Oxytropis lanata*, *Bergenia crassifolia*, and *Astragalus mongholicus*. This study is basically divided into three sections.

**Background of section one and two** - is about isolation, structure determination and anti-parasitic activity of *O. lanata* roots (section one) and *B. crassifolia* roots (section two). Research to find effective compounds from natural products that can be used for parasitic diseases treatments, has grown in recent years. There have many advantages on natural products such as relatively safe, less side effects etc. Furthermore, more effective plant-based products could be contributed to the maintenance of animal health.

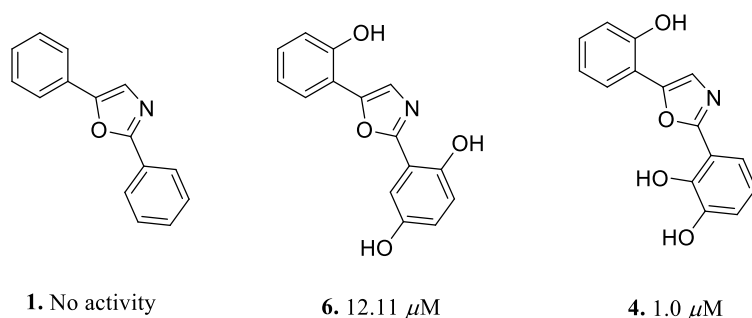
**Background of section three** – is focused on the effects of *A. mongholicus* roots on uterus and ovary of mice (section three). This plant was reported to be effective on some type cancers such as thyroid, breast, ovarian cancer etc. Continuous estrogen stimulation has been known to be cause of cell proliferation, leading to some female disorders and cancers. Estrogens can modulate other nuclear receptor (NR) signaling pathways such as peroxisome proliferator-activated receptors (PPARs). But mechanisms remain poorly understood. NRs including estrogen receptor (ER)  $\alpha$  and PPAR $\alpha$  are associated with abnormal estradiol-related diseases

and cancers of female reproductive organs; indicating that PPAR $\alpha$  and ER $\alpha$  could be potential targets for a variety disease treatment.

**Section one:** *O. lanata* is belonging to Fabaceae, many species are considered important forage and medicinal plants, and meanwhile some species are classified as poisonous plants. In Mongolian traditional medicine, this plant is used to treat bacterial fever, fever from anthrax, bone fractures, bleeding, inflammation, and wounds.

In the result of phytochemical investigations, eleven new compounds that were found in the first time from natural products, 2,5-diphenyloxazole (**1**) and its derivatives (**2-11**), together with six known isoflavonoid derivatives (**12-17**), were isolated from *O. lanata* roots. 2,5-Diphenyloxazole type alkaloids have been found very rare in natural resources and synthetic versions are used in physical fields as a scintillator and liquid laser dye. The other 2,5-diphenyloxazole derivatives (**2-11**) contain one to four hydroxy and/or methoxy groups at their phenyl rings.

The isolated compounds were evaluated inhibition activity against *Trypanosoma congolense*, which is a main cause of African trypanosomiasis, leads to huge economic problems. Some oxazoles (**4-8**) with di- and tri-hydroxy groups at their phenyl rings showed moderate trypanocidal activity (IC<sub>50</sub> 1.00-14.82  $\mu$ M) compared to reference drugs, pentamidine (IC<sub>50</sub> 0.169  $\mu$ M) and diminazene (IC<sub>50</sub> 0.109  $\mu$ M). Among the isolated compounds 2-(2',3'-dihydroxyphenyl)-5-(2''-hydroxyphenyl)oxazole (**4**) showed the highest inhibition activity (IC<sub>50</sub> 1.00  $\mu$ M). The structure and activity relationship was noticed, and the activity was increased depending on hydroxy groups number and their positions at phenyl rings.



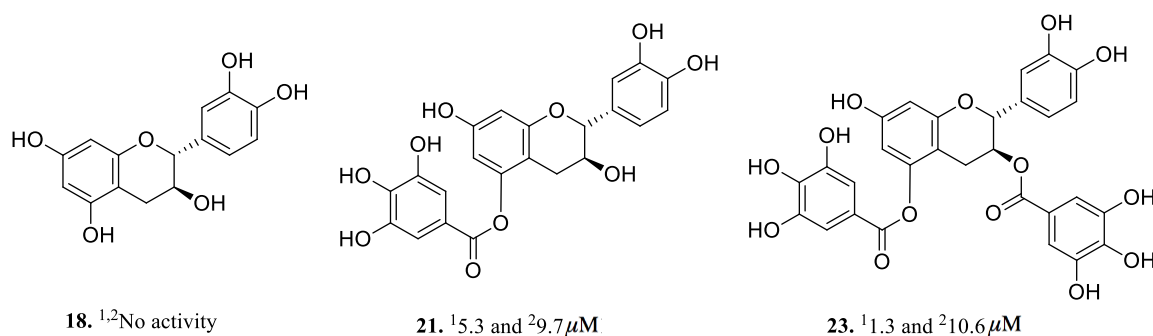
**Figure 1.** The inhibition activity (IC<sub>50</sub>) against *T. congolense* of 2,5-diphenyloxazole and its derivatives

**Section two:** *B. crassifolia* is included in Saxifragaceae family, and used as a valuable medicinal plant in India, Tibet, China, Russia, and Mongolia. For example, in Mongolia, it is

used to treat typhoid and lung fever, stomach and intestine disorders, diarrhea, and in case of lung inflammation. In addition, aerial part of this plant has been widely used as a tea ingredient in Mongolia and Russia.

As a result of phytochemical investigations, two novel compounds, (2R,3S)-3-*O*-*p*-hydroxybenzoyl-5-*O*-galloylcatechin (**22**) and 6-*O*-(3''-*O*-methylgalloyl)arbutin (**32**), together with twenty known flavonoids, including catechins (**18-21**, **23**, **24**), flavonols (**25-29**), arbutins (**30**, **31**, **33**), bergenins (**35-39**), and a tannin (**40**), were isolated from *B. crassifolia* roots. Their chemical structures were elucidated on the basis of spectroscopic analysis, including techniques NMR experiments, and by comparison with the literature.

The compounds were tested the growth inhibition activity against *Babesia bigemina*, *B. bovis*, they are the most prevalent species of Babesiosis. As a result of it, some compounds, **22**, **24**, **33**, **34** and **39**, were showed moderate growth-inhibition effect on anti-babesia activity against *B. bigemina* (IC<sub>50</sub> 9.6 - 13.6 μM), and *B. bovis* (IC<sub>50</sub> 1.3 – 21.6 μM) compared to a reference drug, diminazine acetate (*B. bovis* IC<sub>50</sub> 0.41 μM; *B. bigemina* IC<sub>50</sub> 0.18 μM). Among them, digalloyl catechin derivatives, **22** (IC<sub>50</sub> 1.3 μM) and **24** (IC<sub>50</sub> 1.39 μM) showed strongest activities against *B. bovis*. The activity and structure relationship was clearly observed the inhibition activity was increased depend on number of galloyl groups.

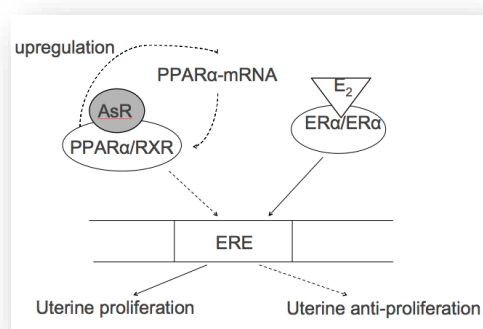


**Figure 2.** The inhibition activity (IC<sub>50</sub>) against <sup>1</sup>*B. bovis* and <sup>2</sup>*B. bigemina* of catechin and its derivatives

**Section three:** *A. mongholicus* that belongs to Fabaceae family is a well-known and widely used herb in traditional East-Asian medicine such as to increase vital body energy, overall vitality etc.

To explore the role of PPARα agonist, female mice were fed a powder diet that included 5% hot water extract of *A. mongholicus* roots (AsR), or 0.1% bezafibrate (used as a positive control) for 56 days. In the results, AsR upregulated the expression of PPARα-mRNA, and downregulated ERα level in the uterus, which led to a significant reduction of uterine mass. In

the ovary, AsR influenced to increases of the PPAR $\alpha$ -mRNA expression level, and 2,4-dienyol-CoA reductase (mDECR) level that has a critical role in mitochondrial  $\beta$ -oxidation. Moreover, the ovary weight increase tendency was observed in AsR group. It could be related to the elevation of mDECR level. In conclusion, *A. mongholicus* root's hot water extract influenced to the uterus anti-proliferation through the way increase PPAR $\alpha$  activation. It also increased  $\beta$ -oxidation in ovary. These findings are rising that AsR could be a candidate for some disorders related to women.



**Figure 3.** Proposed a signaling pathway of AsR in the uterus.

### List of publications

1. Trypanocidal activity of 2,5-diphenyloxazoles isolated from the roots of *Oxytropis lanata*. Orkhon Banzragchgarav, Toshihiro Murata, Gendaram Odontuya, Buyanmandakh Buyankhishig, Keisuke Suganuma, Bekh-Ochir Davaapurev, Noboru Inoue, Javzan Batkhoo and Kenroh Sasaki.  
*Journal of Natural Products*, 2016, 79, 2933-2940.
2. *Astragalus mongholicus* root induces ovarian  $\beta$ -oxidation and suppresses estrogen-dependent uterine proliferation.  
Banzragchgarav Orkhon, Kyoko Kobayashi, Batkhoo Javzan, Kenroh Sasaki.  
Accepted by *Molecular Medicine Reports*. 2018.01.16 (Reference: 194938)