ФИЗИКО-ХИМИЧЕСКАЯ БИОЛОГИЯ

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Review of phytochemical and some biological activity of *Leptopyrum fumarioides* (L.) Reichenb.

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Abstract. *The phytochemical study of Leptopyrum fumarioides started in 2011 and since then 14 volatile compounds have been identified by GC-MS. 7 flavonoids and 4 alkaloids have been isolated and 2 of them have been recognized as new natural compounds. The structures of the natural products have been determined and ascertained by MS, as well as IR, 1D NMR and 2D NMR spectroscopic methods. The antioxidant, antimutagenic, antiproliferative, hepatoprotective, immunomodulatory, and anticancer activity of Leptopyrum fumarioides extracts and some isolated pure compounds were examined. The new alkaloids leptopyrine (**3**) and leptofumarine (**4**) were identified. Leptopyrine (**3**) is a new type of dimer alkaloid of benzylisoquinoline and simple isoquinoline with imine nitrogen. Leptofumarine (**4**) is the first example of aporphine and benzylisoquinoline alkaloid with two ether bridges which have head-to-head and tail-to-tail coupling. This review clearly shows that the phytochemical study of plant species is just beginning and requires more detailed and comprehensive study.*

Keywords: *Leptopyrum fumarioides* (L.) Reichenb., *traditional uses, phytochemistry and biological activities*

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PHYSICOCHEMICAL BIOLOGY

Обзорная статья
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Обзор фитохимических и некоторых исследований биологической активности *Leptopyrum fumarioides* (L.) Reichenb.

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Аннотация. *Фитохимическое изучение Leptopyrum fumaroides началось в 2011 году, с тех пор с помощью ГХ-МС было определено 14 летучих соединений, а также выделены и идентифицированы 7 флавоноидов и 4 алкалоида, 2 из которых были новыми природными соединениями. Строение природных продуктов определено и установлено методами МС-, ИК-, 1D ЯМР- и 2D ЯМР-спектроскопии. Новые алкалоиды получили названия лептопирин (**3**) и лептофумарин (**4**). Лептопирин (**3**) представляет собой новый тип димерного бензилизохинолина и простого изохинолино-*

*вого алкалоида с иминным азотом. Лептофумарин (**4**) является первым примером алкалоида апорфина и бензилизохинолина с двумя эфирными мостиками, которые имеют связи «голова к голове» и «хвост к хвосту». Исследованы антиоксидантная, антимутагенная, антипролиферативная, гепатопротекторная, иммуномодулирующая и противораковая активность различных экстрактов Leptopyrum fumaroides и некоторых выделенных чистых соединений. В данном обзоре наглядно показано, что фитохимическое и биологическое изучение этого вида растений только начинается и требует более детального и всестороннего изучения.*

Ключевые слова: *Leptopyrum fumarioides* (L.) Reichenb., *традиционное использование, фитохимия и биологическая активность*

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INTRODUCTION

Leptopyrum fumarioides belongs to the Ranunculaceae family. *L. fumarioides* is distributed in Siberia, Mongolia, China and North Korea. There are three species of the genus Leptopyrum – *Leptopyrum fumarioides* (L.) Reichenb., *L. tenellum* Raf. and *L. generale* E.H.L. Of these, only *Leptopyrum fumarioides* grows in Mongolia as Khubsugul, Khentii, Khangai, Mongol Daguur, Mongol Altai, Dundad Khalkh, Dornod Mongol and Gobi Altai regions [1–3]. *Leptopyrum* was previously assigned to the genus Isopyrum, but in 1964 Xiao Pei-Keng and Wang Wen-Cai recognized *Leptopyrum* as a separate genus [4]. The genus *Isopyrum* has about 60 species. However, its chemical composition and biological activity have been little studied. So, European researchers isolated and found only alkaloids [5–12].

Compared to the genus *Isopyrum* there is no detailed study on the genus *Leptopyrum*. There is no scientific justification for the use of many medicinal plants, based on the study of the biological functions and activity of their extracts and components. The use of these plants for the manufacture of medicines is based solely on traditions that have been preserved for generations. Therefore, it is important to study the chemical composition, biological functions, and activity of traditional medicinal plants, and confirm their use on a scientific basis [13]. *L. fumarioides* (L.) Reichenb. is an example of one such plant species. However, we could not find any information about the chemical composition and biological activity of the other two species of *Leptopyrum*, so we tried to review the studies of *Leptopyrum fumaroides* and hope that this may help further researchers.

Traditional uses of L. fumarioides. Aerial parts of the plant are used in Mongolian and Tibetan folk medicine for the treatment of fever, typhoid fever, high blood pressure, liver diseases, and dropsy, cardiovascular, gastrointestinal diseases, and for the treatment of various intoxications[1] [14]. In traditional Mongolian medicine, *Leptopyrum fumarioides* and *Hypecoum erectum* L. have similar usage under the same name “Barbad” in variety of traditional medicine for the treatment of liver diseases. *H. erectum* is an ingredient of “Hepamon” drug and in combination with *Salsola colina* it is used for the treatment of liver. The comparative study of “Hepamon” and Silymarin was performed in the liver treatment of rats poisoned with CCl4. After liver intoxication with CCl4, the concentrations of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) in the animals treated with Hepamon were higher than in the animals treated with silymarin [15]. It is worth to mention that *L. fumarioides* extract is one of the components of an anticancer drug in the traditional Chinese folk medicine [16].

Study of chemical compositions of L. fumarioides. The chemical composition of this plant species has been little studied. Previously, it was only found that the herb contains alkaloids, saponins [17, 18], cyanogenic glycosides, and a small amount of ascorbic acid [19, 20].

Alkaloids. From the aerial part of *L. fumarioides* growing in Mongolia, the isoquinoline alkaloid such as known alkaloids protopine (**1**), thalifoline (**2**), and new alkaloids leptopyrine (**3**) and leptofumarine (**4**) were isolated and identified. Leptopyrine (**3**) is a new type of dimer alkaloid of benzylisoquinoline and simple isoquinoline with imine nitrogen (Fig. 1) [21] Leptofumarine (**4**) is the first example of aporphine and benzylisoquinoline alkaloid with two ether bridges which have head-to-head and tail-to-tail coupling[2] (Fig. 1) [21, 22].

Non-alkaloid components. From dichloromethane extract of the aerial part of L. fumarioides, D. Boldbaatar et al. the 14 hydrocarbons were isolated and identified by the GC-MS method, and among them 1-Propoxypentane (**5**), 6,10,13-trimethyltetradecanol (**6**), 8-methylheptadecane (**7**), tetracosane (**8**), heneicosane (**9**), eicosane (**10**), hexadecanoic acid, methyl ester (**11**), phytol (**12**),

[1]Sanchir Ch., Batkhuu J., Boldsaikhan B., Komatsu K. The encyclopedia of Mongolian useful plants. Ulanbataatar: Admon Printing, 2005. Vol. 2. 180 p.

[2]Solongo A. The isoquinoline alkaloids from *Berberis sibirica* Pall. and *Leptopyrum fumarioides* (L.) Reichenb. of Mongolian origin: PhD Thesis. National University of Mongolia, 2019.

2,4-decadienal (**13**), 2-pentadecanone (**14**), 10-methylcosane (**15**), tetradecahydro-7-isopropyl-1,4a-dimethylphenanthren-1-methanol (**16**), pentadecane (**17**), pentadecanoic acid (**18**) (Table). From the n-butanol and aqueous extracts, 7 flavonoids were isolated and identified, such as luteolin-7-O-glucoside (**19**), luteolin 6-C-β-D-glucopyranoside (**20**), apigenin 7-glucuronide (**21**), isovitexin (**22**), naringenin-7-O-glucoside (23), 6-hydroxynaringenin (**24**), naringin (25)[3] (Fig. 2) [23, 24].

Biological activities. Antioxidative, hepatoprotective, antibacterial, antigenotoxic, immunomodulatory and anti-cancer activities of various extracts and some isolated pure compounds of *Leptopyrum fumarioides* were studied.

Antigenotoxic effects or DNA damaging effects. The results of the investigations of *L. fumarioides* showed that its sub extracts and some pure compounds can to prevent catechol-induced DNA damage and possibly play a potent antioxidant role [23]. The various extracts and the most common components of these extracts were tested for their ability to induce DNA damage. It has been shown that Luteloin-7-O-glucoside (9) has no effect on DNA damage up to 10 μg/ml (higher concentrations could not be tested)[4] [24].

The acute toxicity. The acute toxicity of a 40% ethanol dry extract of *L. fumarioides* was studied. The LD50 of this extract was 2500±176 mg/kg. As a result, the extract *of L. fumarioides* was assigned to the group of practically non-toxic substances [25].

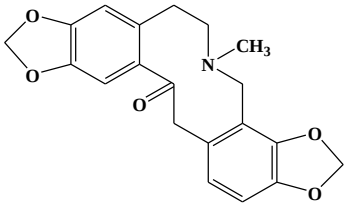
DNA protective effects. The DNA-protective effects of various components of *L. fumarioides* have been studied in mouse lymphoma cells exposed to high concentrations of the DNA-damaging prooxidant catechol. Low concentrations of the three sub-extracts significantly reduced catechol-induced DNA degradation, and the most successful protective agent was flavone luteolin-7-O-glucoside isolated from the n-butanol sub-extract. This compound has been shown to reduce catechol-induced DNA degradation to almost control levels [24].

Immunomodulatory activity. New alkaloids Leptofumarine and Leptopyrine isolated from *L. fumarioides* were evaluated for their effect on macrophages and bone marrow (BM) cells *in vitro*. Our results showed that both alkaloids inhibit cytokine production by LPS-stimulated peritoneal macrophages. Leptopirine suppressed the formation of osteoclasts and osteoblasts, while leptofumarine only affected osteoblastogenesis [22].

Anticancer and antiepileptic activity. L. fumarioides extracts inhibit activity of U-937 GTB in lung cancer cells; in addition, their positive antiepileptic activity has been discovered[5] [24].

Antioxidant activity. The free radical scavenging antioxidant activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) was tested on sub-extracts and *L. fumaroides* extract. The n-butanol sub-extract had the stronger antioxidative activity than both the extract and the other two sub-extracts (the dichloromethane sub-extract was the least effective). The most striking result of the assay was that the isolated flavone, luteolin-7-O-glucoside (9), had a very strong antioxidant activity and were found to have an almost the same IC_{50}-value as the positive control rutin[6] [24].

The screening of DPPH radical scavenging of antioxidant activity, hepatoprotective and antiproliferative activity was carried out. The total alkaloids (**IIIb, IIIc, IIId, IIIe**) were isolated from 4 kg air-dry arial part of *L. fumarioides*. About 4.0 kg of the plant sample was extracted with 95% ethanol 5 times.



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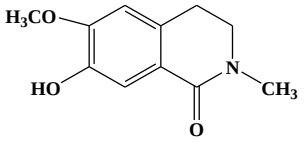

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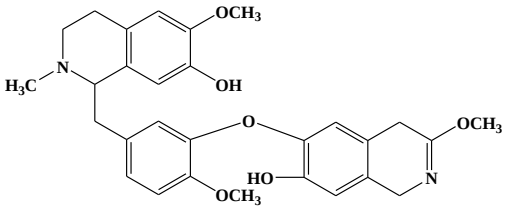

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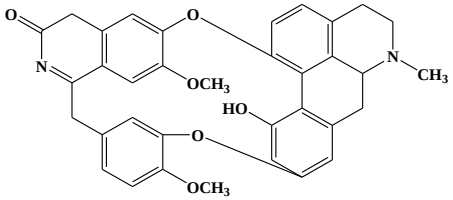

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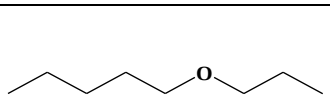
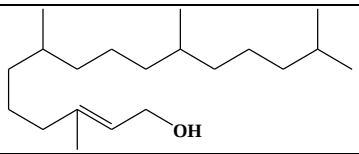
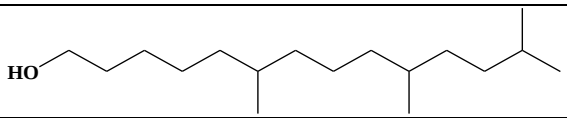
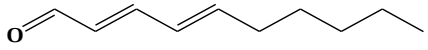
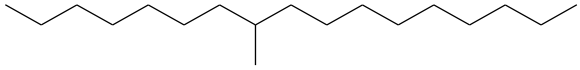
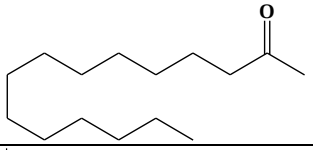
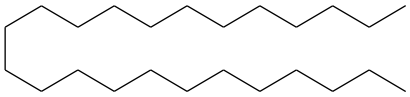
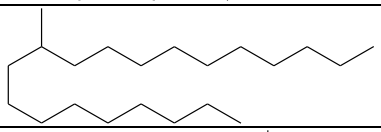
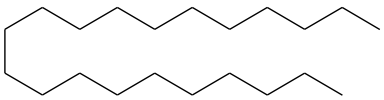
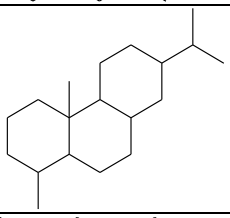
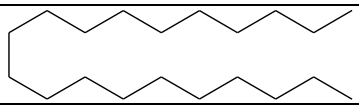
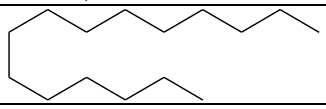
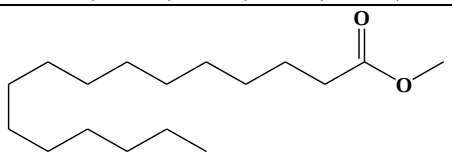
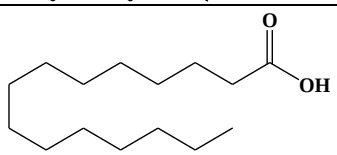
Fig. 1. Structure of alkaloids from *L. fumarioides*

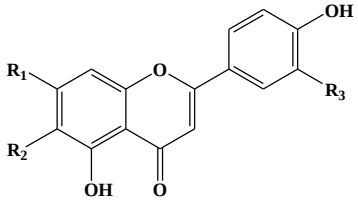
Рис. 1. Структура алкалоидов *L. fumarioides*

[3]Boldbaatar D. Phytochemistry and antimutagenic activity of *Leptopyrum fumarioides* (L.) RCHB.: PhD Thesis. National University of Mongolia, 2018.

The volatile compounds from *L. fumarioides* identified by GC-MS

Летучие соединения *L. fumarioides*, идентифицированные с помощью ГХ-МС

№	Formula	№	Formula
5		**12**	
6		**13**	
7		**14**	
8		**15**	
9		**16**	
10		**17**	
11		**18**	



	19	20	21	22	23	24	25
R_1	-*O*-Glu	-OH	-Glr	-OH	-*O*-Glu	-OH	-*O*-Rp
R_2	-H	-Glp	-H	-Glu	-H	-OH	-H
R_3	-OH	-OH	-H	-H	-H	-H	-H

-*O*-Glu=O-glucoside
-Glr= glucuronide
-Glu=C-glucoside
-O-Rp=rhamnopyranosyl

Fig. 2. Structure of flavonoids from *L. fumarioides*

Рис. 2. Структура флавоноидов *L. fumaroides*

After evaporation 478.25 g of thick extract was obtained (**I**). The extract was dissolved in 5% HCl and purified by hexane 3 times (**IIIa**). The acidic solution was alkalized by 25% NH_4OH to pH 7–8 and extracted with chloroform to obtain 3.06 g of crude alkaloid mixture (**IIIb**). Besides, 25% NH_4OH was added to acidic solution up to pH 9–10 and extracted with chloroform to obtain 2.04 g of crude alkaloid mixture (**IIIc**). If the acid solution was alkalized to pH 9–10 and extracted with chloroform-methanol (4:1), then 0.75 g of polar crude alkaloid mixture (**IIId**) was isolated, while the extraction with butanol gave 163.14 g of crude alkaloid mixture (**IIIe**).

In addition, a 1.5 kg of plant sample was extracted with 70% ethanol 4 times. After evaporation 330.72 g of thick extract was obtained (II). After the use of various organic solvents, the 29.84 g of dichloromethane (IV) fraction, 3.66 g of ethyl acetate (V) fraction, and 65.79 g butanol (VI) fraction were isolated. Alkaloid fractions III_B, III_C, III_D, and III_E were different from each other. All fractions were passed through preliminary screening for hepatoprotective, antiproliferative, and antioxidant activity. According to our study of DPPH radical scavenging screening, all extracts and sub-extracts of *L. fumaroides*, ethyl acetate (IC50 168.58±0.68), and total alkaloids

(IC50 97.84±0.39) showed high antioxidant activity. The antioxidant activity of the extract is considered to be highly active if the IC_{50} is less than 100, moderately active if the IC50 is 100-200, and inactive if it is more than 200. Further study will take up the three various assays such as DPPH radical scavenging antioxidant activity, ABTS antioxidant and Ferric reducing antioxidant power assays on effective fractions [26].

Hepatoprotective activity. Toxic hepatitis had been caused in white rats by subcutaneous injection of 50% oil solution of carbon tetrachloride (CCl4) at the rate of 0.4 ml / 100 g of body weight once a day for four consecutive days. *L. fumarioides* extract at a dose of 200 mg/kg (experimental – therapeutic dose) in the form of an aqueous solution was intragastric administered starting from the second day once a day for 10 days of the experiment. For comparison, a separate group of rats was similarly injected with Holosas in a volume of 1.0 ml/kg of body weight (experimental therapeutic dose). Animals of the control group were injected with an equal volume of purified water according to a similar scheme. After 7, 14 and 21 days from the start of CCl4 administration, the bile-forming and bile-diverting function and the structural state of the rat liver were assessed.

As a result of the study, it was found that the aqueous and 40% ethanol extract *of L. fumarioides* showed choleretic and hepatoprotective effect on rats, as well as stimulating effect on the synthesis and release of cholic acid. It has been shown that *L. fumarioides* extract causes a pronounced inhibition of cholera-forming and choleretic functions of the liver and their destructive changes [25].

According to our study, the hepaprotective effect of extracts and sub-extracts of *L. fumarioides* is induced by t-BHP in cell culture. Ethanol extract, ethyl acetate and butanol sub-extracts have shown a protective effect against cell toxicity. In addition, a more detailed study of the hepatoprotective effects of this species using certain fractions and isolated pure compounds is needed [26].

Antiproliferative effects. The antiproliferative activity of *L. fumarioides* in liver cancer cells Hep G2 was compared with positive control 5-fluoroacyl (an agent that treats many cancers, including colon, rectum, breast, stomach, pancreas, ovaries, bladder, and liver). As a result of this screening, it was found that 29.34% crude ethanol extract and 38.44% crude mixture of alkaloids, and 10.84% dichloromethane sub-extract of *L. fumaroides* showed more pronounced inhibition of the cell growth of liver cancer than the positive control 5-fluoroacyl [26].

CONCLUSIONS

It was found that 4 alkaloids, 7 flavonoids and 14 volatile compounds identified from the *L. fumaroides*. Thus, 25 natural compounds of *L. fumarioides* were isolated and identified.

The antioxidant, antimutagenic, antiproliferative, hepatoprotective, immunomodulatory and anticancer effects of various extracts and some isolated pure compounds of *L. fumarioides* have been studied. In comparison, all of these biologically active studies show that low-polar compounds of plant species have genotoxic and antiproliferative effects. The polar compounds of plant species have antigenotoxic, hepaprotective, and antioxidant effects. Therefore, they are extensively used in the Mongolian as well as Tibetan traditional medicines to relief fever, treat liver diseases and dropsy and also for the treatment of various intoxications. Moreover, it is used as an essential component in the anticancer drug in the Chinese traditional medicine. From this review, it may be possible to provide scientific evidence for the use of traditional medicine. But it may be worthwhile to make a final conclusion based on a more detailed study.

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