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# GC-MS analysis and antibacterial activity of some fractions from *Lagochilus ilicifolius* Bge. grown in Mongolia

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**Abstract:** 3-methyl-1,2,3,4-tetrahydroquinoline (**1**), 4-hydroxyisoquinoline (**2**), 4-(1E)-hydroxy-1-prophenyl)-2-methoxyphenol (**3**), 4-acetoxycinnamic acid (**4**), Songoramine (**5**), and Songorine (**6**) have been determined by GC-MS analysis from the crude alkaloid mixtures ($G_1$) obtained from the aerial parts of *Lagochilus ilicifolius* Bge. grown in Mongolia and comparison of the measured data with those from the literature. The compounds **1**-**6** are described for the first time from *L.ilicifolius*. From these 3-methyl-1,2,3,4-tetrahydroquinoline (**1**) was determined for the first time from natural plants.
In addition, the antibacterial activity of fractions and total alkaloids were evaluated against *Staphylococcus aurous*, *Bacillus subtilis*, *Bacillus cereus* and *Escherichia coli* strains, respectively. The growth inhibition zones against gram-positive *S.aureus*, *B.subtilis*, *B.cereus* and gram negative *E.coli*, strains were observed. Positive results were achieved on 500 µg/disc concentration, but lower results or no active on 100 µg/disc concentration were for the plant extracts, fractions and total alkaloids.

**Keywords:** *Lagochilus ilicifolius Bge., alkaloids, bacterial strains, disc diffusion, GC-MS*

## INTRODUCTION

The genus of *Lagochilus* is belonging to Lamiaceae family with more than 35 species are distributed mainly in central Asia such as Iran, Turkistan, Afghanistan, Russia, Mongolia and China [1-3]. The whole herb of *Lagochilus ilicifolius* has been used in folk medicine for treating haemostatic, inflammation and ulcer in China [4-5]. Also, many scientists revealed the presence of diterpenoids, flavonoids, coumarins, iridoid glycosides, essential oils and polysaccharides in this genus [6-11]. From *Lagochilus hirtus* was isolated alkaloid and identified as a stachydrine [12]. Currently, our botanists found and identified only 3 species of *Lagochilus* from the Mongolian flora. There are *Lagochilus bungii* Benth., *L.diacanthophyllus* (Pall.) Benth., and *L.ilicifolius* Bge. The species *L.ilicifolius* is widely distributed at the Khangai, Mongolian Altai, Middle Khalkha, Depression of Great Lakes, Valley of Lakes, East Gobi, Gobi-Altai, Transaltai Gobi, Alashan Gobi regions [13]. In our previous studies we described isolation and structural elucidation of some phenolic and non polar compounds from the ethanolic and chloroformic extracts of *L.ilicifolius* [14-15]. However, in the present study, we have focused on isolation and structural elucidation of alkaloids and to evaluate against gram-positive *S.aureus, B.subtilis, B.cereus* and gram negative *E.coli* strains for different fractions and total alkaloids from this plant.

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## EXPERIMENTAL

***General experimental procedures:*** The GC-MS analysis were recorded on Hewlett Packard 6890/MSD5973 instrument operating in EI mode at 70 eV. An HP–5 MS column (30 mm×0.25 mm×0.25 µm) was used. The temperature program was 50°C to 300°C at 4°C $min^{-1}$ and 10 min hold at 300°C. Injector temperature was 280°C. The flow rate of carrier gas (He) was 0.8 ml $min^{-1}$. The identities of the alkaloids were confirmed by comparison of the measured data with those from the literature (Table 1).
Column chromatography (CC) was carried out on Silica gel 60 (0.063-0.200 mm), Merck Darmstadt, Germany eluted with petroleum ether-acetone (20:1 to 1:1) mixtures. Thin layer chromatography (TLC) was performed on plates with Kieselgel 60 $F_{254}$ DC-Alufolen (Merck), for solvent system were used $CHCl_3$ -$CH_3OH$, (20:1; 10:1; 9:1; 8:2). Spots were detected under UV light. Visualization of alkaloids were done with Dragendorff`s reagent.
Four pathogenic strains as *Staphylococus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6151), *Escherichia coli* (ATCC 25922) and *Bacillus cereus* were maintained in the Laboratory of Microbiology at the Institute of General and Experimental Biology, MAS in Ulaanbaatar. Control antigen was Ampicillin. The antibacterial activity was determined using a modified Kirby-Bauer agar disc diffusion method. Suspensions of laboratory strains were spread on Petri dishes (Mueller-Hinton Agar (BioMerieuxInc)+5% EBS). For

this purpose 500 and 100 μg/disc concentration of each fractions and total alkaloids of plants were coated on sterile filter paper disc of diameter 8 mm size. The plates were allowed to stay at 4°C for 2 hours before incubation with the test microbial agents at 35°C for 24 hours. The antibacterial activity was assessed on the size of the inhibition zone diameter obtained surrounding the filter paper disc. The incubation zones were measured in millimeters.

***Plant material:*** The aerial parts of *Lagochilus ilicifolius* Bge. were collected from Altai Mountain chains, territory of Khowd aimag, during the flowering period in July, 2012. A voucher specimen is deposited in the Herbarium fund of the Laboratory of natural products chemistry, Institute of Chemistry and Chemical Technology, MAS, Mongolia. The plant material was identified by Prof. Ch.Sanchir, Institute of General and Experimental Biology, MAS.

***Extraction and isolation:*** Air dried and powdered aerial parts (1.29 kg) of *Lagochilus ilicifolius* were extracted exhaustively with 95% ethanol at room temperature 3 times. The combined ethanol extract were evaporated under reduced pressure, acidified with 5% HCl, (pH 1-2), and left overnight at room temperature. Insoluble non-alkaloid materials were removed by filtration, and the filtrate was subjected to n-hexane extraction to eliminate the rest of the non-alkaloid substances. Thus the purified acidic solution was made alkaline to pH 9-10 with 25% $NH_4OH$ and extracted with chloroform 4 times and chloroform-ethanol (4:1) 2 times, respectively. The chloroform and chloroform- ethanol extracts were dried (anhydrous $Na_2SO_4$) and evaporated under reduced pressure then two alkaloid containing fractions controlled and compared by TLC analyses. Finally combined these fractions and to give 0.5371 g of crude alkaloid mixtures (0.062%). The crude alkaloid mixtures was further separated by CC using silica gel, the column (26 cm×2.4 cm) was eluted with petroleum ether-acetone (20:1; 10:1 to 1:1). The elutes were controlled by TLC and visualized by the Dragendorff's reagent. Fractions with similar quality were combined and totally collected 6 sub fractions from D to I. D-77.0 mg, E-36.2 mg, F-34.2 mg, G-142.2 mg, H-313.0 mg and I-49.5 mg, respectively. The fraction G (142.2 mg) was subjected to CC Silica gel 6.0 g, the column (50 cm×1 cm) was eluted with chloroform, chloroform-methanol mixtures 40:1 to 1:1 afforded G1 (73.5 mg) fraction and were investigated on GC-MS analysis.

***Preparation of tested fractions to antibacterial activity:*** The air-dried and powdered aerial parts (317 g) of the *L.ilicifolius* were extracted with 95% ethanol 3 times at room temperature. Ethanol was evaporated under reduced pressure, and obtained 104 g gummy extracts. The gummy extract was suspended in $H_2O$ and filtered, then fractionated by petroleum ether (0.48 g), chloroform (0.53 g), ethyl acetate (1.19 g), n-buthanol (5.64 g) and water remainder (8.23 g), respectively. The ethanolic extract, petroleum ether, chloroform, ethyl acetate, n-buthanolic fractions and water remainder was done antibacterial activity.

## RESULTS AND DISCUSSION

***Phytochemical analysis:*** 3-methyl-1,2,3,4-tetra--hydroquinoline (1), 4-hydroxyisoquinoline (2), 4-((1E)-hydroxy-1-prophenyl)-2-methoxyphenol (3), 4-acetoxycinnamic acid (4), Songoramine (5), and Songorine (6), have been determined using by GC-MS analysis from the crude alkaloid mixtures ($G_1$) obtained of aerial parts of *Lagochilus ilicifolius* Bge. were presented in Table 1. These compounds (1-6) are described for the first time from *L.ilicifolius*. From these 3-methyl-1,2,3,4-tetrahydroquinoline (1) was determined for the first time from native Mongolian plants.

Table 1. Determination compounds using GC-MS analysis from $G_1$ fraction of *L.ilicifolius.*

| Retention time, min. | Compounds | Molecular formula | $M^+$, characteristic ions | % of total ion current | Ref. |
|---|---|---|---|---|---|
| 10.238 | 3-methyl -1,2,3,4-tetrahydro-quinoline (**1**) | $C_{10}H_{13}N$ | 147 (100), 132 (65), 118 (52), 104 (34), 91(25), 77 (15), 63 (8), 51(19), 39 (10) | 1.86 | 16-19 |
| 10.395 | 4-hydroxyisoquinoline (**2**) | $C_9H_7NO$ | 145 (100), 117 (26), 90 (52), 89 (34), 63 (16), 50 (8) | 2.60 | 21 |
| 14.989 | 4-[(1E)-3-Hydroxy-1-propenyl]-2-methoxyphenol, (Coniferyl alcohol) (**3**) | $C_{10}H_{12}O_3$ | 180 (73), 137 (100), 124 (52), 103 (13), 91 (34), 77 (17), 65 (10), 39 (6) | 9.03 | 22 |
| 23.307 | 4-acetoxycinnamic acid (**4**) | $C_{11}H_{10}O_4$ | 206 (0.8), 164 (100), 147 (10), 117 (8), 91 (5), 75 (10), 55 (0.4) | 4.79 | 23-24 |
| 26.123 | Songoramine (Zongoramine) (**5**) | $C_{22}H_{29}NO_3$ | 355 (100), 327 (16), 299 (71), 284 (31), 246 (5), 207 (22), 122 (33), 55 (31) | 3.11 | 25-26 |
| 28.44 | Songorine (Zongorine) (**6**) | $C_{22}H_{29}NO_3$ | 357 (100), 328 (44), 299 (26), 298 (41), 315 (22), 314 (44), 207 (89), 55 (31) | 7.46 | 25-26 |

Herein, we could not find isolation and identification of 3-methyl-1,2,3,4-tetrahydroquinoline from natural sources. 1,2,3,4-tetrahydroquinoline and its derivatives were obtained only for synthesis by different methods [16-18]. Many simple synthetic 1,2,3,4-tetrahydroquinolines have many important pharmacological agents and have been tested as potential drugs [19-20]. The tumor-specific cytotoxicity of many synthetic tetrahydroisoquinoline derivatives in human oral squamous cell carcinoma cell lines were investigated [21]. Coniferyl alcohol is an intermediate in biosynthesis of eugenol, stilbenoids, and coumarin. Gum benzoin contains significant amount of coniferyl alcohol and its esters [22]. Cinnamic acid derivatives have potential antibacterial, antiviral and antifungal properties [23-24]. Songorine and songoramine are diterpenoid alkaloids found in many species of *Aconitum*. Also, diterpene alkaloids occur mainly in the following families as a *Ranunculaceae* (genus *Aconitum and Delphinum*), *Garryaceae* (genus *Garrya*), Escalloniaceae (genus *Anopterus*), *Compositae* (*Inularoyeana*) [25-26].

***Screening of antibacterial activity:*** The antibacterial activity of the ethanolic extract, petroleum ether, chloroform, ethyl acetate, n-buthanolic fractions, water remainder, and total alkaloids were evaluated against 4 strains as a *S.aureus, B.subtilis, B.cereus* and *E.coli* were shown in Table 2. Control antigen was Ampicillin. Numbers indicate the mean diameters of inhibition of triplicate experiments ± standard deviation.

The antibacterial activity was assessed on the size of the inhibition zone diameter obtained surrounding the filter paper disc. Following this incubation diameters of the inhibition zones were measured in millimeters [27-28]. The growth inhibition zones against gram-positive *S.aureus* strains for total alkaloid, ethanolic extract, petroleum ether, chloroform, ethyl acetate, n-buthanolic fraction, and water remainder from

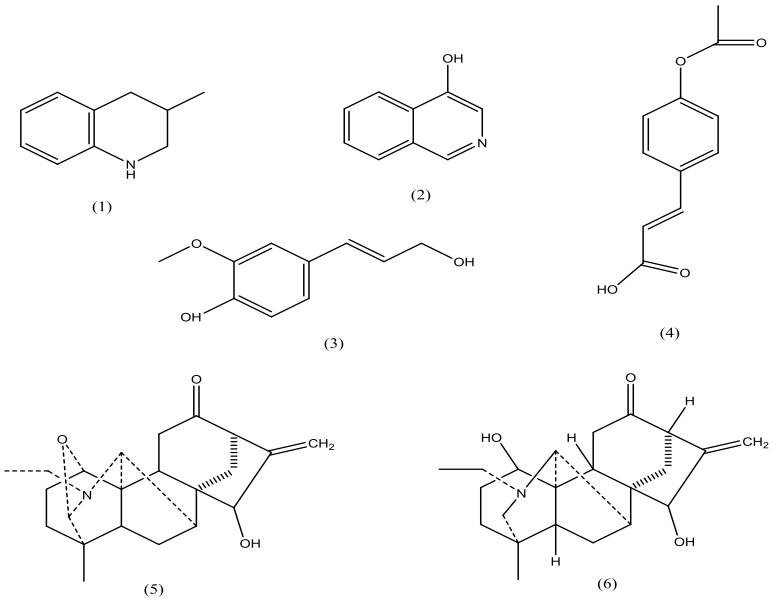


Fig. 1. Structures of the compounds (1-6) determined from *L.ilicifolius*.

Table 2. Evaluation of antibacterial activity of the different samples from *L.ilicifolius*.

| Samples | Concentration of samples, µg/disc | Inhibition zone diameters, mm | | | |
|---|---|---|---|---|---|
| | | *S.aureus* | *E.coli* | *B.subtilis* | *B.cereus* |
| Total alkaloids | 100 | - | - | - | - |
| | 500 | 18 ± 0.02 | 12 ± 0.02 | - | - |
| Ethanolic extract | 100 | - | 2.0 ± 0.1 | - | 5.0 ± 0.1 |
| | 500 | 8.2 ± 0.02 | 20.0 ± 0.02 | - | - |
| Petroleum ether fraction | 100 | 4.0 ± 0.1 | - | - | - |
| | 500 | 20.0 ± 0.02 | 11.0 ± 0.02 | - | - |
| Chloroformic fraction | 100 | - | - | - | - |
| | 500 | 14.0 ± 0.02 | 16.0 ± 0.02 | - | - |
| Ethyl acetate fraction | 100 | - | 3.0 ± 0.1 | 3.0 ± 0.1 | 5.0 ±0.1 |
| | 500 | 12.0 ± 0.02 | 15.0 ± 0.02 | - | - |
| n-buthanolic fraction | 100 | 5.0 ±0.1 | - | - | 5.0 ± 0.1 |
| | 500 | 18.0 ± 0.02 | 8.2 ± 0.02 | - | - |
| Water remainder | 100 | 1.0 ± 0.1 | - | - | - |
| | 500 | 14.0 ± 0.02 | 12.0 ± 0.02 | - | - |
| Ampicilin | 100 | 22.0 ± 0.02 | 16.0 ± 0.02 | 18.0 ± 0.1 | 15.0 ± 0.1 |

- no growth inhibition

*L.ilicifolius* on 500 µg/disc concentrations were showed 18.0±0.02 mm, 8.2±0.02 mm , 20±0.02 mm, 14.0±0.02 mm, 12.0±0.02mm, 18.0±0.02 mm and 14.0±0.02 mm, respectively. The inhibition zones against gram negative *E.coli* strains for total alkaloids, ethanolic extract, petroleum ether, chloroform, ethyl acetate, n-buthanolic fractions and water remainder from *L.ilicifoliuson* 500 µg/disc concentrations were evaluated 12.0±0.02 mm, 20.0±0.02 mm, 11.0±0.02 mm, 16.0±0.02 mm, 15.0±0.02 mm, 8.2± 0.02 mm and 12.0±0.02 mm, respectively. However, the inhibition zones diameter of the following samples from this plants for lower concentrations (100 µg/disc) were showed lower results or no active. Evaluation of antibacterial activity for against *B.subtilis* strains only ethyl acetate fraction was positive and diameter of inhibition zone was 3.0±0.1mm, and for against *B.cereus* strains for ethanolic extract, ethyl acetate and buthanolic fractions *L.ilicifoliuson* 100 µg/disc concentrations were evaluated the same sizes as a 5.0±0.1 mm. Other fractions and total alkaloids from this plants were no active for *B.cereus* strains.

**CONSLUSIONS**

1. This preliminary GC-MS analysis we concluded that *L.ilicifolius* contain 3-methyl-1,2,3,4-tetrahydroquinoline (**1**), 4-hydroxyisoquinoline (**2**), 4-((1E)-hydroxy-1-prophenyl)-2-methoxyphenol (**3**), 4-acetoxycinnamic acid (**4**), Songoramine (**5**), and Songorine (**6**), respectively. 3-methyl-1,2,3,4-tetrahydroquinoline (**1**) was determined for the first time from natural sources.
2. The growth inhibition zones against gram-positive *S.aureus, B.subtilis* and *B.cereus* gram negative *E.coli,* strains for different fractions and total alkaloids from *L.ilicifolius* Bge. were showed a positive results on 500 µg/disc concentration, but on 100 µg/disc concentration were showed lower results or no active, respectively.

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