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PHYTOCHEMICAL INVESTIGATION OF HAPLOPHYLLUM PEDICELLATUM BUNGE ET BOISS AND IDENTIFICATION OF QUINOLINE ALKALOIDS

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Abstract. Haplophyllum pedicellatum Bunge et Boiss is a perennial medicinal plant belonging to the Rutaceae family and widely distributed in Central Asia. The present study was conducted to investigate the phytochemical composition of H. pedicellatum collected from the Bobotog region of Surkhandarya Province, Uzbekistan. The aerial parts were extracted with 80% ethanol under ultrasonic conditions and subsequently fractionated using solvents of increasing polarity. Chromatographic separation of the ethyl acetate fraction yielded several quinoline alkaloids, including furoquinoline derivatives and pyraquinolin-2-one compounds. A previously undescribed alkaloid, designated Pedicin, was isolated and characterized. The roots were extracted with methanol, yielding a total alkaloid content of 0.65%. Further chromatographic purification resulted in the isolation of N-methyl-2-phenylquinolin-4-one and flindersine. Structural elucidation was carried out using UV, IR, ESI-MS, TLC, HPLC, melting point analysis, and NMR spectroscopy. The results demonstrate that H. pedicellatum is a valuable natural source of biologically active quinoline alkaloids with potential pharmaceutical applications.

Keywords: Haplophyllum pedicellatum, Rutaceae, quinoline alkaloids, furoquinoline alkaloids, Pedicin, phytochemistry, medicinal plants, N-methyl-2-phenylquinolin-4-one, flindersine.

Introduction

Medicinal plants remain one of the most important sources of biologically active compounds for the pharmaceutical, cosmetic, and food industries. Among them, species belonging to the genus Haplophyllum occupy a significant position due to their rich content of alkaloids, coumarins, lignans, flavonoids, and essential oils. Numerous investigations have demonstrated that representatives of this genus possess antimicrobial, anti-inflammatory, antioxidant, cytotoxic, and anticancer activities.

Haplophyllum pedicellatum Bunge et Boiss is a perennial herbaceous species characterized by highly branched stems covered with minute glandular structures. The branches arise from the leaf axils and reach a height of 20–50 cm. Leaves are simple, lanceolate, and occasionally three-lobed. Flowers are arranged in dense corymbose or spherical inflorescences. Petals are yellow and broadly ovate, while fruits are dehiscent capsules.

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The plant flowers during May and June, whereas seed maturation occurs in July and August. The species inhabits clay deserts, foothill regions, gravelly sandstone slopes, saline soils, and abandoned lands at elevations ranging from 450 to 1700 m above sea level.

The geographical distribution includes Tashkent, Samarkand, Kashkadarya, and Surkhandarya regions of Uzbekistan, as well as the Kyzylkum Desert, Syrdarya Basin, Pamir-Alay Mountains, and Turkmenistan.

Traditional medicine employs decoctions of the aerial parts for the treatment of toothache and stomach disorders. Combined preparations with Artemisia absinthium have been used against itching and skin diseases. Previous biological studies have also reported inhibitory effects of plant extracts against Ehrlich carcinoma.

Despite its medicinal importance, detailed phytochemical investigations of H. pedicellatum remain limited. Therefore, the objective of the present study was to isolate and identify quinoline alkaloids from this species and evaluate its phytochemical potential.

Literature Review

The genus Haplophyllum comprises more than 70 species distributed mainly throughout Central Asia, North Africa, and the Mediterranean region. Previous investigations have revealed the presence of numerous secondary metabolites, including:

- Quinoline alkaloids;
- Furoquinoline alkaloids;
- Acridone alkaloids;
- Coumarins;
- Flavonoids;
- Lignans;
- Terpenoids.

Quinoline alkaloids are particularly important due to their broad spectrum of biological activities. Several compounds isolated from Haplophyllum species exhibit antibacterial, antifungal, antiviral, antimalarial, and anticancer properties.

Studies on related species such as Haplophyllum perforatum, Haplophyllum tuberculatum, and Haplophyllum obtusifolium have led to the isolation of skimmianine, flindersine, haplopine, dictamnine, and other biologically active alkaloids.

However, information regarding the chemical composition of H. pedicellatum remains scarce. Consequently, phytochemical investigation of this species is of considerable scientific and practical interest.

Materials and Methods

Plant material

Plant samples were collected from the Bobotog region of Surkhandarya Province during the flowering period in June. Botanical identification was performed according to regional floristic keys.

The collected material was air-dried at room temperature and subsequently ground into fine powder.

Extraction of Aerial Parts

The dried aerial parts were extracted using 80% ethanol at room temperature in an ultrasonic extractor.

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The extraction process was repeated 8–9 times to ensure complete extraction of secondary metabolites.

After concentration under reduced pressure, the crude extract was successively partitioned with:

- Petroleum ether;
- Chloroform;
- Ethyl acetate;
- Ethanol;
- Water.

This procedure yielded fractions enriched with compounds of different polarities.

Isolation Procedures

Alkaloids present in the fractions were separated by:

Silica gel column chromatography;

Thin-layer chromatography (TLC);

High-performance liquid chromatography (HPLC);

Recrystallization techniques.

The ethyl acetate fraction yielded a dark precipitate after solvent evaporation. Further purification of this precipitate afforded a yellow crystalline alkaloid.

Repeated chromatographic purification produced another alkaloid with a melting point of 222–224°C, which was designated as Pedicin.

Root Extraction

Root samples were extracted with methanol.

The concentrated extract was treated with 5% sulfuric acid solution to transfer alkaloids into the aqueous phase. Following alkalization with concentrated ammonia, alkaloids were extracted using chloroform.

The total alkaloid content was determined to be 0.65%.

Results

Isolated alkaloids

Phytochemical investigation resulted in the isolation of the following quinoline alkaloids:

1. Furoquinoline alkaloids;
2. Modified furoquinoline derivatives;
3. Pyraquinolin-2-one alkaloids;
4. Pedicin;
5. N-Methyl-2-phenylquinolin-4-one;
6. Flindersine.

The isolated compounds were characterized by chromatographic and spectroscopic methods.

Alkaloids Isolated from Haplophyllum pedicellatum

№	Compound	Class
1	Flindersine	Furoquinoline alkaloid
2	N-Methyl-2-phenylquinolin-4-one	Quinolinone alkaloid
3	Pedicin	Novel quinoline alkaloid

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4	Pyraquinolin-2-one derivatives	Quinolinone alkaloids

Identification of N-Methyl-2-Phenylquinolin-4-one

The isolated compound was obtained as colorless crystals.

Physical and spectral properties:

Molecular formula: $C_{16}H_{13}NO$

Melting point: 144–145°C

ESI-MS: m/z 235.0

UV (nm): 251, 325, 337

IR (cm^{-1}): 1625 (C=O)

NMR Analysis

The ^1H NMR spectrum displayed:

A singlet at δ 3.58 ppm corresponding to the N-methyl group.

A singlet at δ 6.27 ppm assigned to H-3.

Aromatic proton multiplets between δ 7.35–7.51 ppm.

A downfield proton signal at δ 8.46 ppm corresponding to H-5.

The obtained data were consistent with literature values reported for N-methyl-2-phenylquinolin-4-one.

Identification of Pedicin

Pedicin was isolated as a yellow crystalline powder.

Physical properties:

Appearance: yellow powder

Melting point: 222–224°C

Positive reaction with silicotungstic acid reagent

Chromatographic and spectroscopic analyses confirmed its alkaloid nature. The structural features indicated that Pedicin belongs to the quinoline alkaloid group. Further studies are required for complete structural elucidation and biological evaluation.

Discussion

The present investigation demonstrates that Haplophyllum pedicellatum contains a diverse range of quinoline alkaloids. Similar compounds have previously been isolated from other members of the Rutaceae family and are known for significant biological activities.

The isolation of flindersine confirms chemotaxonomic relationships within the genus Haplophyllum. Furthermore, the discovery of Pedicin suggests that the species may contain previously unreported secondary metabolites.

The total alkaloid content of 0.65% indicates that the roots represent an important reservoir of biologically active compounds.

Considering the reported antitumor activity of crude extracts against Ehrlich carcinoma, the isolated alkaloids may contribute to the observed pharmacological effects.

Future investigations should focus on:

- Structural elucidation using 2D NMR techniques;
- Cytotoxic activity studies;
- Antimicrobial screening;

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- Mechanistic investigations of biological activity.

Conclusion

The phytochemical study of Haplophyllum pedicellatum Bunge et Boiss collected from the Bobotog region of Uzbekistan resulted in the successful isolation and characterization of several quinoline alkaloids.

The aerial and underground parts were found to contain furoquinoline alkaloids, pyraquinolin-2-one derivatives, flindersine, N-methyl-2-phenylquinolin-4-one, and a potentially novel alkaloid designated Pedicin.

Spectroscopic analyses confirmed the identity of the known compounds and established the alkaloid nature of Pedicin. The results contribute to the phytochemical knowledge of the genus Haplophyllum and support the potential pharmaceutical value of this species.

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