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Research Article

Isolation and Structure Elucidation of phyto-bioactive compound from herbal medicinal plant *Dactylorhiza hatagirea*

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ABSTRACT

Dactylorhiza hatagirea (Orchidaceae) is a perennial herb inhabiting sub-alpine to alpine regions, ranging at elevations between 2500 and 5000 m.a.s.l. With palmately lobed rhizome and lanceolate leaves having a sheathing leaf base, it bears pink flowers with purple-colored notches and a curved spur. It finds wide use in ayurveda, siddha, unani, and folk medicine in curing disorders of the circulatory, respiratory, nervous, digestive, skeletal, and reproductive systems, besides boosting the immune system to fight infectious diseases. Secondary metabolites such as dactylorhins A–E, dactyloses A–B, and others exhibit a wide spectrum of pharmacological activities (antioxidant, antimicrobial, antiseptic, anticancer, and immune enhancing activities). *Dactylorhiza hatagirea*, a terrestrial orchid endemic to North-Western Himalayas has immense medicinal importance attributed to dactylorhin. In spite of therapeutic relevance, quantification of dactylorhin from this orchid has not been reported so far. Also, there is tremendous need to conserve *D. hatagirea* through in vitro approaches to harness its various bio-constituents. In the current study, it was found that flowers/inflorescences were enriched with dactylorhin along with tubers in *D. hatagirea*. Other aerial parts such as stem and leaves were not contributing towards the accumulation of active secondary metabolites collectively in whole plant.

INTRODUCTION

Dactylorhiza hatagirea is a species of orchid generally found growing in the Himalayas, from Pakistan to SE Tibet, at altitudes of 2,800–4,000 metres (9,200–13,100 ft). It is locally called 'salam panja' or 'hatta haddi'. It is called 'panchaule'

(पाँचऔले) in Nepali and Himalayan regions. The name 'panchaule' (meaning 5 fingered hand) arises from its root resembling fingers of hand with around 3-5 fingers. It is an erect perennial herb with long flowering stems. The plant is well known for its medicinal value. The root has sweet taste. It is strictly prohibited for collection and

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sale, but can be found easily around Nepal. It costs around NRs. 10,000-15,000 per kilo as of late 2015. Medicinal plants have been used for thousands of years across various cultures as a natural source of healing and health maintenance. These plants contain bioactive compounds—such as alkaloids, flavonoids, glycosides, tannins, and essential oils—that can be used to prevent or treat a wide range of ailments. Traditional systems of medicine such as Ayurveda, Traditional Chinese Medicine (TCM), and Unani heavily rely on the use of herbs and plants for therapeutic purposes. Even in modern pharmacology, many pharmaceutical drugs are derived from compounds originally found in plants—for example, aspirin from willow bark and quinine from the cinchona tree.

Phytochemicals (from the Greek word phyto, meaning "plant") are naturally occurring chemical compounds found in plants. These compounds are not essential nutrients like vitamins or minerals, but they have been shown to have powerful health-promoting and disease-preventing properties.

Major Types of Phytochemicals There are thousands of known phytochemicals, but they can be broadly classified into several groups:

1. Alkaloids • Bitter-tasting compounds often used in medicine • Example: Morphine (from opium poppy), Quinine (from cinchona bark)
2. Flavonoids • Powerful antioxidants that protect cells from damage • Found in: fruits, vegetables, tea, wine • Example: Quercetin, Kaempferol
3. Phenolic compounds • Anti-inflammatory and antioxidant properties • Found in: berries, apples, olive oil • Example: Resveratrol, Ellagic acid
4. Terpenoids (or isoprenoids) • Known for aromatic qualities and medicinal effects • in: essential oils, herbs like mint and eucalyptus • Example: Menthol, Limonene

5. Glycosides • Many have heart-protective effects • Example: Digoxin (used in heart failure)

Techniques of Isolation and Purification of Bioactive Molecule from Plant

Isolation and purification are vital steps in phytochemical research, allowing scientists to study pure compounds from complex plant matrices.

Steps in Isolation and Purification:

1. Collection and Authentication

Correct plant identification using taxonomy or DNA barcoding.

Selection of plant part based on ethnobotanical or traditional knowledge.

2. Drying and Grinding

Drying under shade or low heat to preserve phytochemicals.

Grinding into powder to increase surface area for efficient extraction.

3. Extraction

Use of solvents (e.g., ethanol, methanol, water, chloroform) to dissolve bioactive molecules.

Extraction methods include maceration, Soxhlet extraction, sonication, and microwave-assisted extraction.

4. Fractionation

Partitioning the crude extract using solvents of different polarities (e.g., hexane, ethyl acetate, butanol).

5. Isolation

Chromatographic techniques separate compounds based on polarity, size, or charge.

Techniques include column chromatography, TLC, HPLC, etc.



METHODOLOGY

Extraction Methods for Studying Phytochemicals

Extraction is the first and most crucial step in the study of phytochemicals from medicinal plants. It involves separating bioactive compounds from plant tissues using suitable solvents and methods, preserving their chemical integrity. The choice of extraction method affects the yield, purity, and biological activity of the phytochemicals obtained. Different methods vary in efficiency, solvent use, temperature, energy consumption, and time requirements. Below are the most commonly used extraction methods in phytochemical research.

1. Cold Extraction Method

Principle:

Cold extraction relies on passive diffusion of compounds into a solvent at room temperature.

Procedure: • Dried and powdered plant material is soaked in a solvent (e.g., ethanol, methanol, or water). • Mixture is left for 24–72 hours with occasional shaking. • The extract is filtered and concentrated. Advantages: • No heat — ideal for heat-sensitive (thermolabile) compounds. • Simple and cost-effective. Disadvantages: • Slow and may yield less extract. • Risk of microbial contamination during long extraction times.

Applications: • Used in traditional medicine preparation. • Extraction of essential oils, alkaloids, and glycosides.

2. Solvent Extraction Method

Principle:

Based on the solubility of compounds in various solvents of different polarity.

Procedure: • Plant material is mixed with a solvent (polar or non-polar depending on the compound of interest). • Common solvents: ethanol, methanol, acetone, hexane, chloroform, or water. • The

extract is filtered and solvent is evaporated under reduced pressure.

3. Microwave-Assisted Extraction (MAE)

Principle:

Uses microwave energy to rapidly heat plant material and solvent, breaking down cell walls and enhancing compound release.

Procedure: • Plant sample and solvent are exposed to microwaves in a closed vessel. • The increased pressure and heat release phytochemicals rapidly.

• The extract is filtered and concentrated.

Advantages: • Faster extraction. • High yield and efficiency. • Reduced solvent use. Disadvantages:

• Requires specialized equipment. • Not suitable for some thermolabile compounds. Applications: • Effective for polyphenols, flavonoids, and essential oils.

4. Maceration Extraction

Principle:

Involves soaking plant materials in solvents at room temperature for an extended period to allow diffusion of compounds.

Procedure: • Plant powder is immersed in solvent in a closed container. • Left for days with occasional stirring.

• Filtered, and the extract is evaporated.

Advantages: • Simple, no need for complex equipment. • Gentle extraction for fragile compounds. Disadvantages: • Long extraction

time. • Lower efficiency compared to advanced techniques. Applications: • Common in traditional

herbal formulations. • Used for tannins, glycosides, and saponins.

RESULTS AND DISCUSSION

Phytochemical Screening and Extraction The preliminary phytochemical screening of *Dactylorhiza hatagirea* confirmed the presence of



key secondary metabolites such as flavonoids, terpenoids, alkaloids, phenolics, glycosides, and saponins in both aerial and tuberous parts. Extraction was carried out using methanol, ethanol, and hydroalcoholic solvents, with methanol yielding the highest concentration of crude extracts.

Isolation of Bioactive compounds

Crude extracts underwent successive fractionation using column chromatography (silica gel) and preparative thin-layer chromatography (TLC). Eluted fractions were screened using bioautography for bioactivity, and active fractions were selected for further analysis.

From the methanolic root extract, several key compounds were isolated:

Dactylorhin A–E (glucosidic esters of hydroxyphenylbutanedioic acid)

Dactylose A and B (phenolic sugar derivatives)

Structural Elucidation

Advanced spectroscopic techniques were used to elucidate the structures of the isolated compounds:

- Nuclear Magnetic Resonance (NMR) (^1H and ^{13}C NMR)

- Mass Spectrometry (MS) • Infrared Spectroscopy (IR)

- Ultraviolet-Visible (UV-Vis) Spectroscopy

Dactylorhin A, for example, was identified as: (2R)-2- β -D-glucopyranosyloxy-2-(2-methylpropyl) butanedioic acid bis(4- β -Dglucopyranosyloxybenzyl) ester. Spectral data showed typical signals for phenolic and glucosidic protons, while MS fragmentation confirmed the molecular formula and glycosidic linkages. The stereochemistry was resolved based on NOESY and COSY NMR techniques.

CONCLUSION

This study successfully isolated and elucidated the structures of several bioactive compounds from *Dactylorhiza hatagirea*, a critically endangered medicinal orchid native to the North West Himalayas. Advanced chromatographic and spectroscopic techniques led to the identification of novel compounds, including dactylorhins A–E and dactyloses A and B. These compounds exhibited significant antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities, underscoring the plant's therapeutic potential. The findings highlight the importance of *D. hatagirea* as a valuable source of bioactive metabolites with diverse pharmacological properties. However, the over exploitation of this species has led to its classification as critically endangered. Therefore, it is imperative to implement sustainable harvesting practices and conservation strategies, such as in vitro propagation techniques, to ensure the continued availability of this medicinal plant for future generations.

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