Original article

Isolation of lupenone (18-Lupen-3-one) from Roscoea purpurea root extract

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Abstract:

Background: Endangered plant “Kakoli” is important component of Ashtwarga group of plants and anti-aging Ayurvedic preparations. Due to limited supply of original plant, official substitutes and common adulterants are being used by drug manufacturers. There is a need to identify a marker compound that could differentiate original plant from substitutes and common adulterants. Objective: To isolate and characterize the marker compound from roots of this plant. Material and methods: The extract of plant root was prepared in methanol and marker compound was isolated from methanol extract through column chromatography by using silica gel (60–120 mesh size) in glass column (1000mm x 50mm). The compound was obtained in fractions numbered 990-1550 and isolated by cutting and pooling of TLC plate of compound having Rf = 0.52 by the use of mobile phase toluene: ethyl acetate: formic acid (9.5: 0.5: 0.1 v/v/v). Compound was characterized by using IR, NMR, Mass and UV spectroscopy. Results: The methanol extract was blackish brown in color and showed the presence of alkaloids, terpenoids, phytosterols, flavonoids, phenolics and amino acid. The isolated compound was found to be colorless terpenoid needle with m.p. 168-171°C; [α]D +62.8° (c 1.0,CHCl3). Spectral analysis confirmed presence of lupenone. Conclusion: In present study lupenone was isolated for the first time from Kakoli. None of adulterants and substitutes of Kakoli are reported to have lupenone hence can be used as marker for identification as well as differentiation of the plant from official substitutes and common adulterants.

Keywords: Kakoli; Lupenone; Isolation; Marker; Zingiberaceae

Introduction

Since ages India has been blessed with the wealth of miraculous medicinal plants. Ayurveda was not in text form and was passed on from teacher to taught by recitation and memory. This tradition passed on from generation to generation till the Ayurveda was compiled and found its rightful place at the end of the fourth Veda, the Atharvaveda. Plants and plant products have been recognized in Rigveda and Atharvaveda for cure of a number of ailments 1. Due to different attitude of rulers at different times and due to advent of other therapies including Unani and Tibbi, a good deal of Ayurvedic literature was lost. This led to a decline in the glory of Indian medicine in as much as a number of effective remedies were lost. In their place a number of worthless drugs of doubtful origin came in which did not have the curative properties and thus the good name of the Indian system of medicine got overshadowed 2. The same happened with Ashtwarga group of plants and formulations in which these plants were used as important components. “Ashtawarga” constituting a group of eight plants (Jivaka, Rishbhaka, Meda, Mahameda, Kakoli, Kshirakakoli, Riddhi and

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Vriddhi) form an important component of a number of Ayurvedic preparations including well renowned preparation “Chyawanprash” 3,4.

Roscoea is a very well-known tuberous perennial herb that is grown for its eye-catching flowers and tolerance of wet and shady conditions. There are 22 recognized species out of which eight are endemic to China. Roscoea purpurea also known as Roscoea procera (Wall.) locally renowned as kakoli, red gukhra, dhawankholika, karnika, ksheera, vayasoli and vaysasha etc. is native of Nepal and Himalayas 5.

Tubers of R. purpurea are known to exhibit immunomodulatory activity 6, antidiabetic activity 7 and are having, anti-oxidant, anti ageing effect that elevates overall health status of a well being 8-10. A negligible amount of work has been done on development of chemical markers that can be used for identification and differentiation of authentic plants from cheap substitutes, official substitutes (Ashwgandha or Kali Musali) and common adulterants.

**Experimental Section**

**Chemicals and Plant material**

The root samples of Roscoea purpurea were procured from Himachal Pradesh and authenticated by Central Instrumentation Facility, National Botanical Research Institute, Lucknow having authentication letter no. NBRI/CIF/524/2016. Roots were washed, shade dried and stored in air tight container. All the solvents and reagents used in the study were of analytical grade.

**Preparation of extract**

Roots of Roscoea purpurea were coarsely powdered and defatted with petroleum ether followed by extraction with methanol through continuous hot extraction process. It was filtered, evaporated to obtain a semisolid mass and extracts were then stored in vacuum desiccator.

**Phytochemical screening**

Preliminary phytochemical screening was performed for the detection of phyto-constituents like alkaloids, glycosides, steroids, terpenoids, flavonoids, tannins, phenolics, saponins, carbohydrates, proteins and amino acids 11-13.

**Isolation of marker**

About 8.4g of methanol extract was added in methanol and silica gel (60–120 mesh size) to form slurry that was mixed and dried on water bath resulting into a free flowing powder. Silica gel (675g) suspended in n-hexane was poured into the glass column (1000mm x 50 mm) to give rise to silica bed. Silica bed was saturated and slurry was charged and allowed to stand overnight for uniform bed packing. Elution was started with n-hexane followed by an increase in polarity of solvent. Fractions were collected with optimum flow rate of 10ml/min. Fractions were pooled on the basis of thin-layer chromatography (TLC) profile. TLC of fractions was performed using different solvents selected by hit and trial method. Elution with the solvent system toluene yielded a pool of four compounds with Rf 0.34, 0.52, 0.67, 0.81 on TLC plates by the use of mobile phase toluene: ethyl acetate: formic acid (9.5: 0.5: 0.1 v/v/v). Single compound was isolated by cutting and pooling of TLC plate of compound having Rf = 0.52. The compound was obtained in fractions numbered 990-1550 and purified by re-crystallization with methanol. The fraction was kept in a refrigerator to get the crystallized compound 14,15.

**Characterization of isolated compound**

Isolated compound was characterized by using different chemical test, melting point and spectral analysis (IR, NMR, Mass, and UV spectroscopy).

**Melting point**

Melting point of isolated compound was noted using melting point apparatus.

**Ethical clearance:** Not applicable (-NA-)

**Results and Discussion**

The methanol extract was blackish brown in color and showed the presence of alkaloids, terpenoids, phytosterols, flavonoids, phenolics and amino acid on preliminary phytochemical screening. The isolated compound was found as colorless needle after crystallization from CHCl3-MeOH; melting point 168-171°C (melting point of standard compound is 166-172°C); [α]D +62.8° (c 1.0, CHCl3) and showed the presence of terpenoids.

**Extractive value:** The methanol extractive value was found to be 2.97% (w/w).

**Spectral Analysis of Isolated Compound**

**IR**

The IR spectra show the peaks in cm⁻¹ at 3430.61, 3079.67 (-C=CH2 stretching), 2924.33-2853.80 (aliphatic C-H stretching), 1738.33 (carbonyl C=O stretching), 1645.49 and 1376.91 (C=C stretching non-conjugated), 1461.47 (CH3 deformation), 1261.61 (-C-H in plane deformation), 1095.98, 1022.07 (C-O bending), 885.93 (=CH2 deformation), 801.33 (=CH3)n- skeleton; confirm the skeleton of 18-Lupen-3-one (Lupenone) (Fig. 1).
The spectra also shows two triplet of H-29 α & β proton at δ 4.44 and 4.71 with (J = 1.64 Hz) (Fig. 4) which confirms the structure of lupenone.

**Mass Spectra**

The mass spectra of compound showed a molecular ion peak at m/z 425 (M + H>). It also showed fragmentation peaks at 109, 119, 135, 142, 154, 168, 191, 207, 237, 245, 265, 281, 296, 317, 333, 344, 372, 403 & 417 which are being in agreement with the proposed structure of Lupenone (Fig. 5).

**Structure and Molecular Formula of Isolated Compound**
The molecular formula of isolated molecule is $C_{30}H_{48}O$ that is confirmed by IR and mass spectral data available in literature and its structure is $(1R, 3aR, 5aR, 5bR, 7aR, 11aR, 11bR, 13aR, 13bR) - 3a, 5a, 5b, 8, 8, 11a-hexamethyl - 1 - (prop - 1 - en - 2 - yl) octadecahydro - 1H – cyclopenta [a] chrysene - 9(5bH) - one or lupenone.

Structure of isolated compound lupenone

There is a sharp decline in human expertise capable of recognizing the variety of medicinal plants. Modern Ayurvedic physicians are dependent on herbs collected by local traders or readymade Ayurvedic drugs in the market. Industry is forced to accept the herbs brought by suppliers and traders on their terms without any enquiry and efforts. They are not aware about the authentic sources. The professional raw material collector is unable to meet the increasing demand and it leads to adulteration with other plants that degrade the quality of drug and credibility of the Ayurvedic system of medicine. It has also been found that the adverse drug reactions are not due to the intended herb, but rather due to the presence of an unintended herb. Sometimes, it is very difficult to trace these adulterations because medicinal plant dealers have discovered the ‘Scientific’ methods to adulterate a high quality material in such a way that is difficult to analyze without microscopic and chemical analysis. Ashtawarga plants being an important part of many Ayurvedic formulations are also available in limited amount and likely to be substituted by cheap easily available substitutes or adulterants. The isolated compound lupenone has not been reported in official substitute of Kakoli like Ashwagandha (root) (Withania somnifera) or Kali Musali (root) (Corculigo orchioids Gaerth). Hence Lupenone can act as a chemical marker for differentiating the substitution of authentic plants with official substitutes or adulterants.

Lupenone is known for medicinal properties such as anti inflammatory, anthelmintic and anti-cancer activities. Lupenone has been studied for its inhibitory activity on inflammation under in vitro conditions and in animal models of inflammation. The effective scavenging activity of the Lupenone against ROS proved its potential antioxidant property. Another study showed that Lupenone inhibited $\alpha$-glucosidase ($\alpha$-Glu) in vitro and having antihyperglycemic activity. Lupenone have been reported to inhibit protein tyrosine phosphatase 1B (PTP 1B) which appears to be an attractive target of new drug development for type 2 diabetes and obesity. Therefore the isolated compound, lupenone from Roscoea purpurea root extract is having significant therapeutic potential. Presence of Lupenone has not been reported in its common adulterants/substitute Withania Somnifera.

**Conclusion**

In the present study authors isolated lupenone from the roots of Roscoea purpurea using chromatographic techniques and characterized it by using different spectroscopic techniques. This isolated compound may be used as chemical marker for identification of this plant as well as differentiation of authentic plant from substitutes and common adulterants. Isolation of Lupenone will enable the regulatory authorities to use it for quality control of costly Ayurvedic formulations.

**Conflict of Interest:** Nil

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**Authors’s contribution:**

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