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

**G Kaur**, **V Gupta**, **P Bansal**, **S Kumar**, **RK Rawal**, **RG Singhal**

**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. 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. 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

---

1. G Kaur, UCER, Baba Farid University of Health Sciences, Faridkot, Punjab
2. V Gupta, UCER, Baba Farid University of Health Sciences, Faridkot, Punjab
3. P Bansal*, UCER, Baba Farid University of Health Sciences, Faridkot, Punjab
4. S Kumar, Central Ayurveda Research Institute for Respiratory Disorders (CARIRD), Patiala, Punjab
5. RK Rawal, ISF College of Pharmacy, Moga, Punjab
6. RG Singhal, Shobhit University, Meerut, Uttar Pradesh

**Correspondence to:** Dr. Parveen Bansal, Joint Director, University Centre of Excellence in Research, Baba Farid University of Health Sciences, Faridkot, India
E-mail: bansal66@yahoo.com, ucer_bfuhs@rediffmail.com
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. \(^5\)Roscoea purpurea\(^6\) also known as Roscoea procera (Wall.) locally renowned as kakoli, red gukhra, dhawankholika, karnika, ksheera, vayasoli and vaysasha etc. is native of Nepal and Himalayases\(^5\). Tubers of \(R.\) purpurea are known to exhibit immunomodulatory activity \(^9\), 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 \(R.\) 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 \(R.\) 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 phytoc-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 \(R_f\) 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 \(R_f = 0.52\). 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 CHCl\(_3\)-MeOH; melting point 168-171°C (melting point of standard compound is 166-172°C); \([\alpha]_d +62.8^\circ\) (c 1.0, CHCl\(_3\)) 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=CH\(_3\) 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 (CH\(_3\) deformation), 1261.61 (-C-H in plane deformation), 1095.98, 1022.07 (C-O bending), 885.93 (=CH\(_2\) deformation), 801.33 (-CH\(_3\)) skeleton; confirm the skeleton of 18-Lupen-3-one (Lupenone) (Fig. 1).
Fig. 1. IR spectra of isolated compound.

Fig. 2. $^1$H-NMR spectra of isolated compound lupenone.

$^1$H-NMR (400 MHz, CDCl$_3$, $J$/Hz) $\delta$: 0.70 (3H, s, CH$_3$), 0.77-0.92 (8H, m), 1.12-1.18 (2H, m), 1.20 (9H, s, 3CH$_3$-23, 24 & 26), 1.25 (9H, s, 3CH$_3$-27, 28 & 29), 1.28-1.58 (6H, m), 1.59-1.65 (4H, m), 1.75-1.79 (2H, bd), 1.95-2.05 (1H, m), 2.28-2.32 (1H, m), 2.35 (1H, s), 4.44 (1H, t, $J$ = 1.64 Hz, H-29$\alpha$), 4.71 (1H, t, $J$ = 1.64 Hz, H-29$\beta$) as shown in Fig. 2. $^1$H-NMR spectrum shows characteristic signals of three singlet for seven methyl groups at $\delta$ 0.70, 1.20 and 1.25 (Fig. 3). The spectra also shows two triplet of H-29 $\alpha$ & $\beta$ proton at $\delta$ 4.44 and 4.71 with ($J$ = 1.64 Hz) (Fig. 4) which confirms the structure of lupenone.

Fig. 3. $^1$H-NMR spectral expansion of isolated compound lupenone from $\delta$ 0.6-2.6.

Fig. 4. $^1$H-NMR spectral expansion of isolated compound lupenone from $\delta$ 4.0-5.0.

**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).

Fig. 5. Mass spectra of isolated compound lupenone.

**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.

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\(^{18-20}\). 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\(^{21,22}\). 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\(^{23}\). 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

**Acknowledgement:** This work was supported by the National Medicinal Plants Board, Ministry of AYUSH, Govt. of India, New Delhi, India [Grant numbers R&D/CH-01/2012].

**Source of Funding:** National Medicinal Plants Board, Ministry of AYUSH, Govt. of India, New Delhi, India [Grant numbers R&D/CH-01/2012].

**Authors’s contribution:**

Idea owner of this study: P Bansal, S Kumar

Study design: V Gupta, P Bansal, S Kumar

Data gathering: G Kaur, V Gupta, P Bansal, RK Rawal

Writing and submitting manuscript: G Kaur, V Gupta, S Kumar,

Editing and approval of final draft: P Bansal, RK Rawal, RG Singhal

---

Structure of isolated compound lupenone
References:
1. Khosa RL. Pharmacognosy and Ayurveda. *Journal of Science Research in plants and medicine* 1981; 2 (4): 113-115.
2. Koul S, Chaudhary A and Khosa RL. Pharmacognosy: its role in solving the crisis of Ayurvedic drugs of doubtful identity. *International Journal of Analytical, Pharmaceutical and Biomedical Sciences* 2015; 4 (5): 23-26.
3. Raval SS, Mandavia MK, Sanghani JM, Mahatma MK and Golakya BA. Separation and identification of phytochemicals from *Roscoea procera*, an ingredient of Ashtavarga. *Indian Journal of Agricultural Biochemistry* 2015; 28 (2): 143-149.
4. Virk, JK, Gupta V, Kumar S, Singh R, Bansal P. Ashtawarga plants - suffering a triple standardization syndrome. *J. Tradit. Complement. Med.* 2017; 7: 392-399.
5. Misra A, Srivastava S, Verma S and Rawat AKS. Nutritional evaluation, antioxidation studies and quantification of polyphenolics in *Roscoea purpurea* tubers. *BioMedical Central Research Notes* 2015; 8: 1-7.
6. Sahu MS, Mali PY, Waikar SB and Rangari VD. Evaluation of immune modulatory potential of ethanolic extract of *Roscoea proceru* rhizomes in mice. *Journal of Pharmacy and Bioallied Sciences* 2010; 2 (4): 346–349.
7. Bairwa R, Basyal D, Srivastav B. Study of anti-diabetic and hypolipidemic activity of *Roscoea purpurea* (zingiberaceae). *International Journal of Institutional Pharmacy and Life Sciences* 2012; 2 (4):130–137.
8. Singh G, Rawat GS, Ethnomedicinal survey of Kedarnath wildlife sanctuary in Western Himalaya, India. *Indian Journal of Fundamental and Applied Life Sciences* 2011; 1 (1): 35–36.
9. Singh AP. Ashtavarga—Rare medicinal plants. *Ethnobot Leaflets* 2006; 10: 104–108.
10. Kaur G, Gupta V. Bansal P. *Roscoea purpurea*: A comprehensive monograph. Mauritius, Lap Lambert Academic Publishing, 2018.
11. Banu KS, Cathrine L. General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Sciences* 2015; 2 (4): 25-32.
12. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. London, Chapman & Hall, 1988.
13. Sohail T, Ferheen S, Imran H, Yaqeen Z, Rehman A and Khan R. Phytochemical and antibacterial screening of different fractions of root part of *Ipomea turpethum*. *Bangladesh Journal of Medical Science* 2018; 17(1): 93-97.
14. Virk JK, Bansal P, Kumar S, Singh R and Rawal RK. Isolation and Quantification of Chemical Marker of *Polygonatum Verticillatum*: First Report. *Current Traditional Medicine* 2016; 2 (3): 216–224.
15. Gupta R, Aggarwal M, Baslas RK. Chromatographic separation and identification of various constituents of essential oil from bulb of *Malaxis acuminate*. *Indian perfume* 1978, 22 (4): 287-288.
16. De Smet PAGM. Adverse effects of herbal drugs. In De Smet PAGM, Keller K, Hansel R, Chandler RF, ed. Toxicological outlook on the quality assurance of herbal remedies. Verlag Heideberg, Springer, 1992: 1-7.
17. Afq SH. A Comparative Introduction of the Unani and Tibetan Medical Traditions. New Delhi, Ayur Vijnana, Rashtriya Sanskrit Sansthan, 1999.
18. Ovesna Z, Vachalkova A, Horvathova K and Tothova D. Pentacyclic triterpenoic acids: new chemoprotective compounds Minireview. *Neoplasma* 2003; 51 (5): 327-333.
19. Roy SK, Ali M, Sharma MP and Ramachandram R. New pentacyclic triterpenes from the roots of medicinal plants available from medicinal plants of India. *Die Pharmazie* 2001; 56 (3): 244-246.
20. Libby P, Schonbeck U. Drilling for Oxygen Angiogenesis Involves Proteolysis of the Extracellular Matrix. *Circulation Research* 2001; 89 (3): 195-197.
21. Na M, Kim BY, Osada H and Ahn JS. Inhibition of protein tyrosine phosphatase 1B by lupeol and Lupenone isolated from *Sorbus commixta*. *Journal of Enzyme Inhibition and Medical Chemistry* 2009; 24: 1056–1059.
22. Mohamed IE, El- Nur EBE. Choudhary MI, Khan SN. Bioactive natural products from two sudanese medicinal plants diospyros mespiliformis and croton zambesicus. *Records of Natural Products* 2009; 3: 198–203.
23. Xu F, Wu H, Wang XP. RP-HPLC Characterization of Lupenone and β-Sitosterol in Rhizoma Musae and evaluation of the Anti-Diabetic Activity of Lupenone in Diabetic Sprague-Dawley Rats. *Molecules* 2014; 19: 14114-14127