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Isolation of Chemical Marker from *Roscoea purpurea*: First Report

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ABSTRACT

Background: *Roscoea purpurea* also known as Kakoli is an endangered species of "Ashtwarga" group which suffer a lot of confusion in Ayurvedic literature in accordance with the identification & authentication. Due to less availability and high medicinal value of Kakoli plant, adulteration and substitution was done by drug manufacturers. Due to which the faith in herbal drugs decreases. **Objective:** Thus for quality standardization and identification of Kakoli containing herbal formulations the present study was designed to isolate chemical marker compound using chromatographic and spectral analysis techniques. **Method:** The methanol extract of root samples of plant was prepared and phytochemical screening was performed for common phytoconstituents. Marker compound was isolated from methanol extract through column chromatography using silica gel in glass column. Single compound was isolated by cutting and pooling of TLC plate of compound having R_f value 0.66 by using mobile phase chloroform: ethyl acetate: methanol (2.5: 3.0: 4.5 v/v/v) and purified by recrystallization with methanol. Isolated compound was characterized by using melting point and spectral analysis (IR, NMR and Mass). **Results:** The methanol extract was blackish brown in color and showed the presence of flavonoids, steroids, terpenoids and alkaloids. The isolated compound was found to be white crystalline powder with melting point ranges from 180-185ºC. Spectral analysis and chemical test confirmed the presence of *Sitostanol caffeate* (SC). **Conclusion**: In present study *sitostanol caffeate* was isolated for the first time from *Roscoea purpurea* and can be used as marker for identification and differentiation of the plant from substitutes.

INTRODUCTION

Ayurveda is an ancient system of medicine which includes the use of herbal preparations along with advice on diet, exercise, sleep and hygiene. Ayurvedic medicine was practiced for thousands of years before anyone invented the placebo controlled experiment. According to leading scientists, research in Ayurveda is fragmented and often not deep in its own foundational theories or in its interface with modern science. It is still in an infancy and a cultural change is needed in current practitioners.[1] Plants and plant products have been recognized in Rigveda and Atharvaveda for cure of a number of ailments.[2] 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 system of medicine. 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.[3] The same happened with Ashtwarga group of plants that are mainly found in natural habitats of the north-western Himalaya. "Ashtawarga" constituting a group of eight plants (Jivaka, Rishbhaka, Meda, Mahameda, Kakoli, Kshirkakoli, Riddhi and Vriddhi) form an important component of a number of Ayurvedic preparations including well renowned preparation "Chyawanprash".[4]

Roscoea purpurea locally renowned as *kakoli*, a perennial herb belonging to family Zingiberaceae,[5] is native of Nepal and Himalayas that extended northwards up to china.[6] Tubers of *R. purpurea* are tasty, nutritious tonic, aphrodisiac that exhibit immuno-modulatory and antidiabetic activity. It also restores health, strengthens immunity system and rectifies defects in anabolism or body growth processes and work as antioxidant in the body.[7,8] Presence of various phytochemicals and active phenolic compounds makes the plant highly significant for therapeutic purposes. But according to International Union for Conservation of Nature [IUCN] because of varied climate, over-exploitation, deforestation, habitat loss, the plant has been included in the red list of endangered species etc. To overcome problem of non-availability of endangered species, Department of AYUSH, Govt. of India has permitted the use of official substitutes in Ayurvedic formulations on the basis of Ayurvedic concepts.[9,10] The official substitutes further encouraged manufacturers for adulteration by other substandard and spurious raw drugs because the Department of AYUSH, GOI lacks the quality control tools (marker compound) needed for identification of authentic plant. Thus,

for quality standardization of herbal products and its formulations, there is a need to isolate chemical marker from Kakoli by using different chromatographic as well spectral techniques.

MATERIALS AND METHODS

Plant material

The root samples of *Roscoea purpurea* were procured from Himachal Pradesh and authenticated by Central Instrumentation Facility (CIF), National Botanical Research Institute, Lucknow having authentication letter no. NBRI/CIF/535/2017 dated 04/01/2017. Root samples of the plant were washed, shade dried and stored in airtight container.

Chemicals

In the present study, all the reagents and solvents used were of analytical grade. For isolation of markers, precoated aluminum-backed TLC plates with 0.2 mm layer of silica gel 60 F_{254} (20 cm × 10 cm) manufactured by E. Merck (Germany) were purchased from local authorized dealer.

Preparation of extract

Roots of *Roscoea purpurea* were powdered coarsely and defatted with petroleum ether followed by continuous hot extraction process with methanol. The resulting extract was then filtered, evaporated to obtain a semisolid mass and was then stored in vacuum desiccator for further use.

Phytochemical screening

For the detection of phytoconstituents like alkaloids, steroids, terpenoids, glycosides, flavonoids, tannins, phenolics, saponins, carbohydrates, proteins and amino acids preliminary phytochemical screening was performed.[11-13]

Isolation of chemical marker

About 8.4g of methanol extract was mixed with methanol and silica gel having pore size 60–120 mesh to form slurry which was then dried on water bath to form a free flowing powder. Silica gel (675g) suspended in *n*-hexane was poured into the glass column having dimensions 1000 mm x 50 mm to give rise to silica bed. Saturated silica bed was allowed to stand

overnight for uniform bed packing. Elution was started with *n*-hexane followed by an increase in polarity of solvent and fractions were collected with optimum flow rate of 10ml/min. Thin-layer chromatography (TLC) of collected fractions were performed using different solvents selected by hit and trial method and similar fractions were pooled together on the basis of TLC profile. Elution with the solvent system ethyl acetate: methanol 20:80 yielded a pool of major five compounds with R_f 0.22, 0.49, 0.66, 0.80 and 0.96 along with mixture of other compounds on TLC plates by using chloroform: ethyl acetate: methanol (2.5: 3.0: 4.5 v/v/v) as mobile phase. Single compound was isolated by cutting and pooling of TLC plate of compound having R_f value 0.66 and purified by recrystallization with methanol. The fraction was kept in a refrigerator to get the crystallized compound.[13-14]

Characterization of isolated compound

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

- **Ultra Violet Spectroscopy:** Perkin Elmer Hitachi 330 (lambda 15 UV/VIS) spectroscopy
- **Infrared Spectroscopy:** Multispoke FT-IR synthesis monitoring system, Perkin Elmer Germany
- **Nuclear Magnetic Resonance Spectroscopy:** Bruker Avance II Spectrometer at 500 MHZ
- **Mass Spectroscopy:** Model Q-ToF Micro Waters equipped with Electrospray Ionization.

Melting point

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

RESULTS

Physical evaluation of extract

The methanol extract was blackish brown in color.

Phytochemical screening of extract

Methanol extract subjected to preliminary phytochemical analysis was confirmed the presence of flavonoids, steroids, terpenoids and alkaloids.

Characterization and identification of isolated compound

Physicochemical description

Isolated compound was found as white crystalline powder after crystallization from $CHCl_3$-MeOH.

Chemical test

The compound was shown positive test of steroids.

Melting point

Melting point of the compound was found to be 180-185ºC (lit. 179-186°C).

Spectroscopic data

Infrared (IR) spectra of Isolated Compound

Very strong overlapping bands in the IR spectrum, which appear in the region of 3438.4 cm^{-1}, were assigned to the different O–H vibrations. Both the C–H stretching modes of aromatic and aliphatic moiety were found at 2857.3, 2927.2 and 2957.5 cm^{-1}. The strong intensities band at 1734.1 (IR) was assigned to the C=O stretching modes of the caffeate ester with Stigmastanol (sitostanol) phytosterol. In addition, the bands of the medium and strong intensities at 1458.7 cm^{-1} were assigned to the C=C stretching modes of the acyclic chain and benzene moiety which confirm the skeleton of *sitostanol caffeate* (SC) (Figure 1).

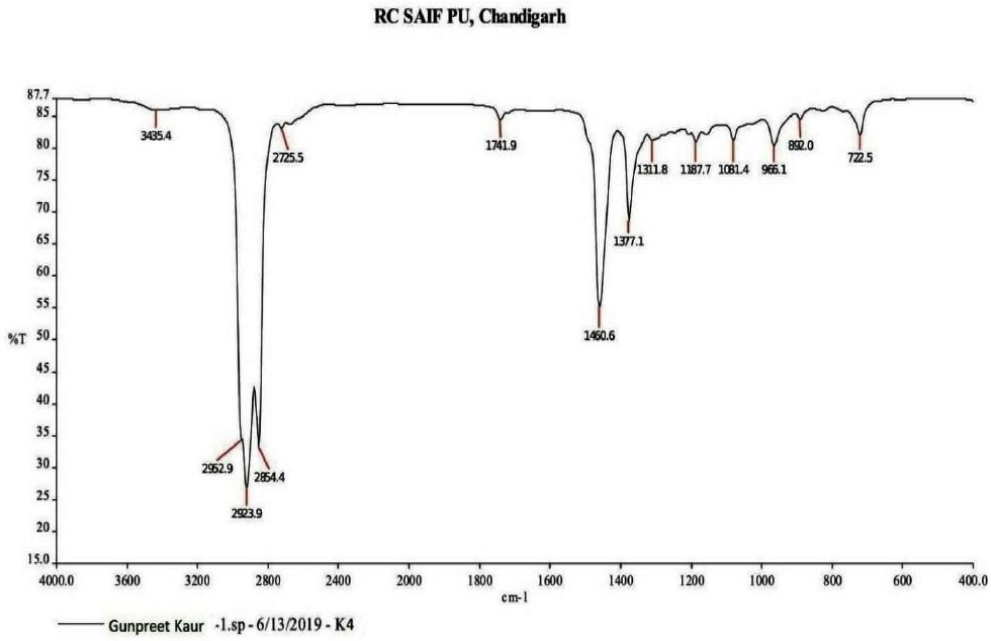

Figure 1: IR spectra of isolated compound *sitostanol caffeate*.

Nuclear Magnetic Resonance (NMR)

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.69-0.94 (m, 10H), 1.07-1.44 (m, 12H), 1.60 (m, 4H), 1.67 (t, 1H, *J*=6 & 6.5), 2.00-2.05 (m, 4H), 2.17-2.21 (m, 7H), 2.29-2.35 (m, 4H), 3.53-3.75 (m, 4H), 4.04-4.05 (m, 1H), 4.13-4.16 (m, 1H), 4.18-4.25 (m, 2H), 4.27-4.30 (m, 1H), 5.25 (bs, 1H, OH), 5.34 (bs, 1H, OH), 7.16 (dd, 1H, *J*=8.5 & 2.5), 7.35 (s, 1H), 7.52-7.53 (dd, 2H, -CH=CH-), 7.69-7.71 (m, 1H). ^{1}H-NMR spectrum shows phenolic –OH at δ 5.25 & 5.35, the characteristic signal of two proton of olefinic proton of -CH=CH- in the form of doublet at 7.52 and 7.54, respectively which confirm the structure of *sitostanol caffeate* (Figure 2).

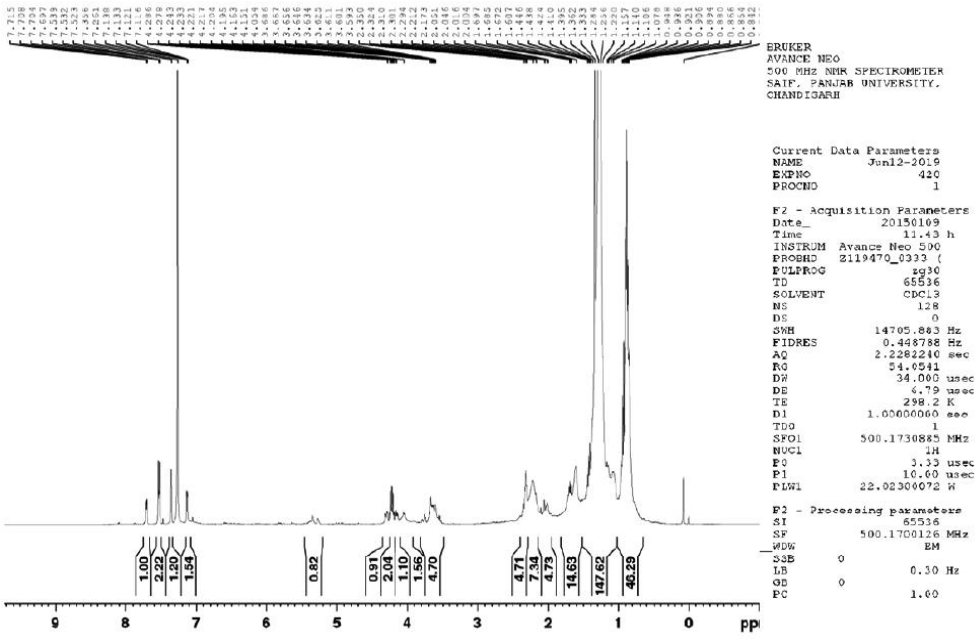

Figure 2: NMR spectra of isolated compound *sitostanol caffeate*.

Mass Spectra

The molecular ion peaks were found at m/z 578.43 (M^+) and 601.43 ($M+Na^+$) in mass spectra of the isolated compound. The mass spectra also showed parent ion peak at 601.43 ($M+Na^+$) which was being in agreement with the proposed structure of *sitostanol caffeate* (Figure 3).

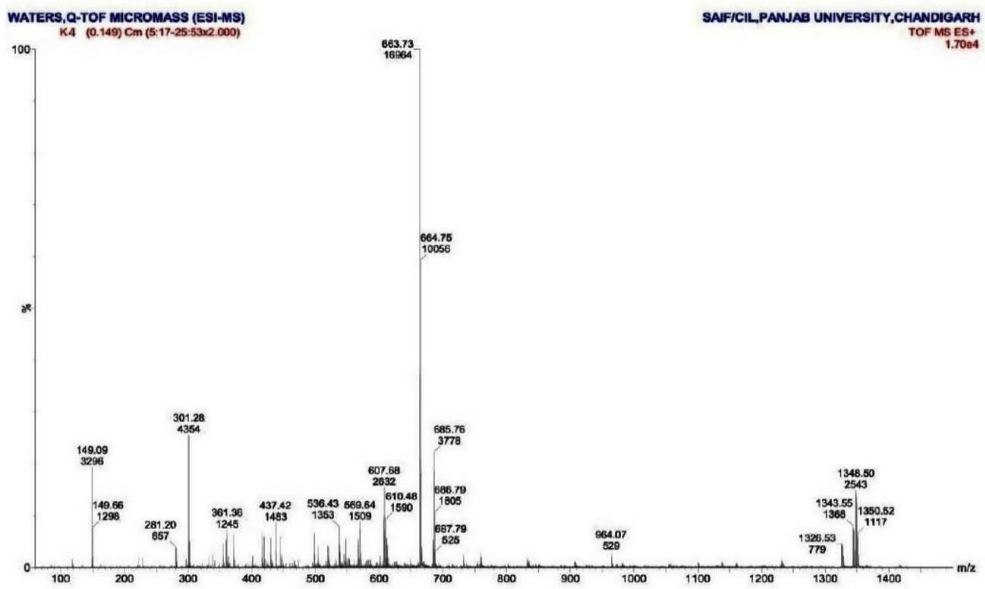

Figure 3: Mass spectra of isolated compound *sitostanol caffeate*

Structure and Molecular Formula of Isolated Compound

The molecular formula of isolated molecule *sitostanol caffeate* is $C_{38}H_{58}O_4$ that is confirmed by IR, mass spectra and NMR data. Its IUPAC name is (E)-(5S,8R,9R,10S,13R,14R,17R)-17-((2R,5R)-5-ethyl-6-methylheptan-2-yl)-8,10,13-trimethylhexadecahydro-1H-cyclopenta[a] phen-anthren-3-yl3-(3,4-dihydroxyphenyl) acrylate (Figure 4).

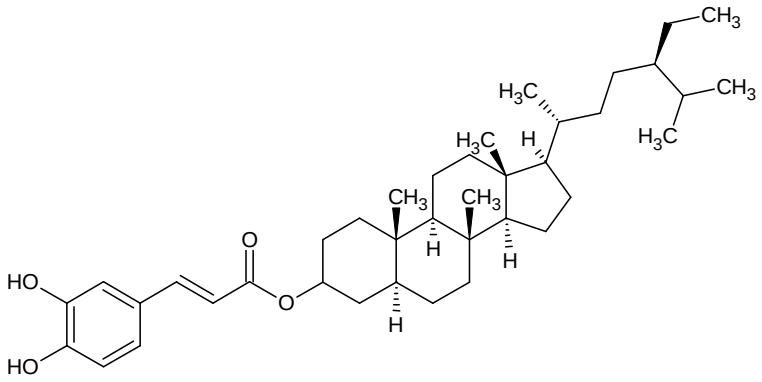

Figure 4: Structure of isolated compound *sitostanol caffeate*

DISCUSSION

Sitostanol caffeate (SC) is made from vegetable oils or the oil from pine tree wood pulp and is then combined with canola oil. It is mainly used for prevention of heart disease and treatment of high cholesterol. *Sitostanol caffeate* is an ingredient in Benecol margarine and some salad dressings. The U.S. Food and Drug Administration (FDA) allow the manufacturers of products that contain *Sitostanol caffeate* or related plant chemicals (stanol esters) to claim that the product lowers the risk of getting Coronary Heart Disease (CHD). S*itostanol caffeate* and other plant stanol esters along with a diet low in saturated fat and cholesterol might reduce the risk of CHD by lowering blood cholesterol levels. Although there is plenty of evidence that *sitostanol caffeate* does lower cholesterol levels, so far there is no proof that long-term use actually lowers the risk of developing CHD.[15-17] As the market price of *Sitostanol caffeate* is very high $3000/g (approximately) so, it will be difficult for commercial manufacturers to replace Kakoli plant with *sitostanol caffeate* just to claim the presence of Kakoli.[17-22] Hence the isolated chemical marker compound that is *sitostanol caffeate* can be used for identifying substitutes in high cost formulations claiming the use of this rare medicinal plant Kakoli.

CONCLUSION

In the present study authors isolated *sitostanol caffeate* from roots of *Roscoea purpurea* using column chromatography and Thin Layer Chromatography. As per our knowledge, this is first report on chromatographic method of isolation of SC from natural source that is *Roscoea purpurea* plant. This compound can be used as a chemical marker by regulatory authorities for identification of kakoli plant from substitutes.

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CONFLICT OF INTERESTS

No conflict of Interests

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