Original Article

Isolation and characterization of quinine from *Polygonatum verticillatum*: A new marker approach to identify substitution and adulteration

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Abstract

Polygonatum verticillatum (Mahameda) is an important ingredient of Ashtawarga and Ayurvedic formulations. Nowadays, it comes under the category of endangered plants due to large scale and indiscriminate collection of wild material. To overcome the scarcity, substitutes of Mahameda are also commonly used in market. These additives are different from the authentic plant by Ayurvedic and pharmacological theory of drug action, thereby resulting in substitution/adulteration. Substitution is a critical issue in isolation and quantification of the therapeutically active ingredients that can be used as markers in the identification of substitution/adulteration. Methanolic extract of the rhizomes of *P. verticillatum* was subjected to preliminary phytochemical screening for the detection of phytoconstituents, followed by column chromatography for isolation of the marker. The column was first eluted with pure hexane, and polarity of the solvent was gradually increased. A total of 1180 fractions were collected and pooled on the basis of thin-layer chromatography profile. The single compound was isolated and confirmed by chemical test, melting point, spectral analysis, and comparison with literature. Phytochemical screening of the extract shows the presence of alkaloids, flavonoids, carbohydrates, terpenoids, and phenolics. A pure white crystalline powder was isolated by column chromatography which was characterized as (6-methoxyquinolin-4-yl-8-vinylquinuclidin-2-yl) methanol, i.e. Quinine. The isolated compound, Quinine, was identified as a novel compound in Mahameda as it has not been reported in the genus *Polygonatum,* till now. It can be used as a marker for the identification of substitution/adulteration and standardization of *P. verticillatum.*

Key words: Adulterants, column chromatography, marker compound, *Polygonatum verticillatum,* substituents

INTRODUCTION

Around 5000 years ago, *Polygonatum* species has been practiced in Chinese and European health-care system. *Polygonatum verticillatum* is a flowering perennial plant of the family *Liliaceae* and is commonly known as Mahameda in Hindi and Whorled Solomon's Seal in English. Mahameda is a deciduous, erect plant of *Polygonatum* genus having bell-shaped greenish-white flowers, mid-green leaves, and red fruits. In India, Mahameda is found in temperate Himalayas from Kashmir (at an altitude of 2000–3600 m asl) to Sikkim (at an altitude of 2600-4000 m asl), Himachal Pradesh and Uttarakhand (1600–3500 m asl). Mahameda

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is a vital ingredient of Ashtawarga group and numerous Ayurvedic formulations such as Chyawanprash, Vachadi Taila, Astavarga Churna, Chitrakadi Taila, Mahakalyan Ghrita, Mahamayura Ghrita, Mahapadma Taila, Jivaniya Ghrita, Brahini Gutika, Vajikaran Ghrita, Indrokta Rasayan, etc.[1] Due to extensive usage, the demand of Mahameda is progressively increasing which leads to large scale and indiscriminate collection of wild material and ultimately to scarcity of the authentic source. Currently, Mahameda comes under the category of endangered plants.[2,3]

To meet out the market pressure and to overcome the scarcity, substitutes of Mahameda and other Ashtawarga plants are commonly in use. Mahameda has been officially substituted by Shatavari,[4] which in turn is substituted by Shakakul Mishri. However, Mahameda has different chemical constituents, pharmacological actions, and Ayurvedic parameters of drug action from its substitutes. This situation directly leads to substitution/adulteration, resulting in further decline of cultivation of Mahameda and other rare plants.[2,5] Thus, it is a critical issue to identify and isolate the therapeutically active ingredients of Mahameda that can be used as markers for the identification of unauthorized substitution/adulteration.

Traditionally, Mahameda is known to be effective against emaciation, senility, pain, pyrexia, weakness, burning sensation, phthisis, and pulmonary affections and also has other significant effects such as tonic, galactagogue, emollient, aphrodisiac, insecticidal, and leishmanicidal.[2] Therapeutic actions of Mahameda are mainly due to the presence of steroidal saponins and polysaccharides in the rhizome.[3] Rhizomes of Mahameda have been proven for anti-oxidant,[6] antispasmodic, antidiarrheal,[7] antipyretic,[8] tracheorelaxant, anti-inflammatory,[9] antimicrobial,[10] antinociceptive, diuretic,[11] and antimalarial potential.[12] Rhizomes of Mahameda are known to contain phytoconstituents such as lysine, serine, aspartic acid, threonine, diosgenin, β-sitosterol, sucrose, glucose,[1] micronutrients (Zn, Fe, Pb, Cu, Ni, Cd, Cr, Co, Sb and Mn), macronutrients (Na, Ca, and K), and essential life nutrients (proteins, fats, carbohydrates, and ascorbic acid).[13] Few compounds have been isolated from the rhizomes of *P. verticillatum* which include lectins,[14] 5-hydroxymethyl-2-furaldehyde,[12] diosgenin, santonin,[6] 2-hydroxybenzoic acid, and β-sitosterol.[9] The present study was designed to isolate and identify other important phytoconstituents of Mahameda, to assist as markers in identifying adulteration.

MATERIALS AND METHODS

Plant material

Rhizomes of *P. verticillatum* were collected from Himachal Pradesh, India from a cultivated source in August–September 2014 and authenticated by the cultivator vide letter number no. HRG/Testimonial-NMPB/02/2015-2016. Further authentication of the plant material was done at the Central Instrumentation Facility, National Botanical Research Institute, Lucknow, India vide Ref. No: NBRI/CIF/524/2016. The plant material was shade dried (<40°C), coarsely powdered, and stored in an air tight container.

Chemicals

All the chemicals and reagents used during the study were of analytical grade and were purchased from different companies such as Qualikems, Finar, and Merck. Infrared (IR) was recorded on a PerkinElmer Fourier transmission infrared, and nuclear magnetic resonance (NMR) spectra were recorded using $CDCl_3$ as solvent on Bruker Avance II 400 NMR spectrometer at Panjab University, Chandigarh, and ultraviolet (UV) spectra were recorded on a high-resolution UV-visible spectrometer.

Extraction of *Polygonatum verticillatum*

Coarsely powdered rhizomes of *P. verticillatum* were first defatted with petroleum ether and then extracted with methanol, by continuous hot extraction process using a Soxhlet apparatus. The extract was filtered and concentrated by distillation to obtain a semi-solid mass and percentage yield was calculated. The extract was kept in a desiccator for further use.

Phytochemical screening of extract

The extract of *P. verticillatum* was subjected to preliminary phytochemical screening for the detection of various phytoconstituents, namely, alkaloids, glycosides, steroids, terpenoids, anthraquinone flavonoids, tannins, phenolic compounds, saponins, carbohydrates, proteins, and amino acids,[15,16] as given in Table 1.

Isolation of marker compound

The extract was subjected to column chromatography for the isolation purpose.[17]

Preparation of slurry

Rhizome extract (8.5 g) of the plant was dissolved in a minimum amount of methanol, and sufficient quantity of silica gel (60–120 mesh size/0.120–0.250 mm particle size)

Table 1: Phytochemical screening of extract

Class of compound	Presence
Alkaloids	+
Proteins	−
Saponins	−
Terpenoids	+
Flavonoids	+
Phenolics	+
Tannin	−
Carbohydrates	+

+: Present, −: Absent

was added, with trituration, for uniform mixing. After appropriate mixing, the slurry was dried on a water bath to get a free flowing material.

Packing of column (wet packing)

680g of silica gel (0.120–0.250 mm particle size) was suspended in hexane and poured into the glass column (60 × 40). After saturation of the bed, drug slurry was loaded into the column, and the column was allowed to stand overnight for uniform bed packing.

Elution of column

Column was first eluted with pure hexane and polarity of the solvent was increased gradually by adding chloroform (9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7, 2:8, and 1:9) to pure chloroform, followed by ethyl acetate-methanol to pure methanol, further followed by increasing amount of methanol in the same manner. A total of 1180 fractions, each of 100 ml, were collected with flow rate of 4 ml/minute, as per prescribed standard flow rate. Mobile phase for thin-layer chromatography (TLC) was standardized by hit and trial method, and TLC was performed using solvent system having different polarity. Fractions having similar TLC profiles were pooled to give the major fraction. A total of 58 pooled subfractions were collected from the column. A white-colored crystalline compound was obtained from the fractions numbered 830–965. Purification of the isolated compound was done by recrystallization through methanol. The fractions were kept in a refrigerator to get the crystallized compound. Confirmatory analysis was carried out through chemical tests, melting point, ^{1}H-NMR, ^{13}C-NMR and compared with literature.

Characterization of isolated compound

Chemical tests

The extract was treated with a few ml of dilute HCl, filtered, and subjected to the following tests:

Dragendorff's reagent test

To 2–3 ml of filtrate, 0.5 ml of Dragendorff's reagent was added. Formation of orange-brown precipitate revealed the positive test for alkaloids.

Mayer's reagent test

To 2–3 ml filtrate, 0.5 ml of Mayer's reagent was added; formation of a cream-colored precipitates revealed the positive test for alkaloids.

Melting point

The melting point of the crystallized compound was determined using a melting point apparatus.

Spectral analysis

Different spectroscopic techniques such as IR, NMR, mass, and UV spectral analysis were used to identify the structure of the isolated compound.

Thin-layer chromatography of isolated compound with extract

The aim of thin-layer chromatographic study was to develop such a method which can separate the marker of the extract. Out of tried mobile phases, n-Hexane: ethyl acetate (4:6 v/v) showed the best separation of the isolated compound in the extract.

RESULTS

Physical evaluation of extract

A dark brown-colored semi-solid mass was obtained, and the percent yield was found to be 14% (w/w).

Phytochemical screening of extract

Phytochemical screening of the extract is shown in Table 1, which indicated the presence of various phytoconstituents.

Identification of isolated compound

Physicochemical characterization

The isolated compound was found as a white crystalline powder, having a percent yield of 0.015% and the melting point in the range of 173°C–175°C, which was similar to that of the melting point of standard Quinine (174°C–175°C).

Chemical test

The isolated compound showed positive test of flavonoids and alkaloids.

Spectral analysis

Infrared of isolated compound

The IR spectra (KBr) (ν/cm) show the peaks at - 3206.22 O-H str (H-bonded), 2967.03-2901.27 C-H str (alkane, alkene, aromatic), 1620-1432 C = C str (aromatic, alkene), 1473-1432 CH_2 bend (alkane, cycloalkane), 1299-1358 C-N str (cyclic amine), 1243-1080 C-O str (ether), 1080-1009 C-O str (alcohol), 980-575 = C-H bend (out of plane), and 780-718 CH_2 bend-rocking type (alkane). These characteristic peaks confirm the presence of Quinine skeleton in the molecule [Figure 1].

^{1}H-nuclear magnetic resonance spectrum

^{1}H NMR ($CDCl_3$, 400 MHz) δ in ppm: 0.00 (TMS proton), 1.24-1.30 (CH_2 of azabicyclo ring), 2.00-2.04 (CH_2 of piperidine ring), 1.60 (-C-O-H), 3.94 (ether -O-CH3), 4.96-5.11 (=CH2), 6.99-7.89 (isoquinoline). Obtained data [Figure 2] clearly signify the presence of Quinine as the isolated compound.

Mass spectrum

The mass spectra of the isolated compound (LC-MS) showed remarkable peaks at (m/z): 136.20 (99.99%), 189.10 (6.26%), 117.10 (5.7%), 79.10 (4.1%), and 324.41 (1.3%, M^+). These details are in agreement with the proposed structure and strictly comply with the mass spectrum of standard Quinine [Figure 3].

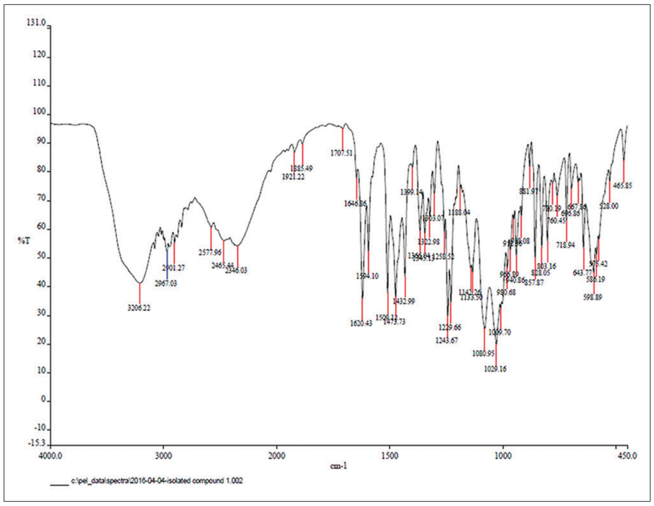

Figure 1: Infrared spectrum of the isolated compound

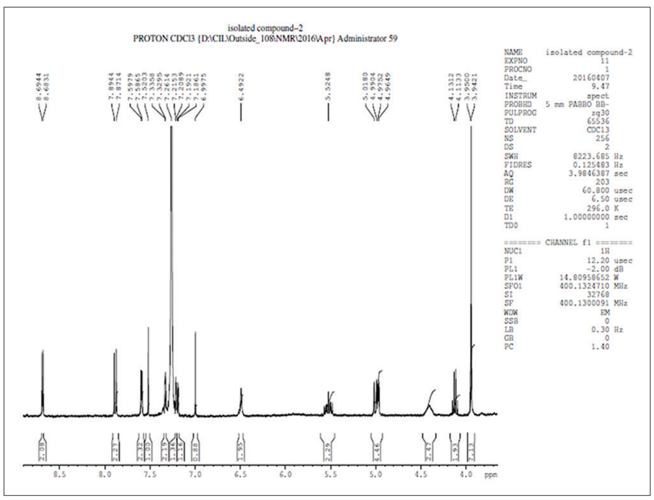

Figure 2: Nuclear magnetic resonance spectrum of the isolated compound

Ultravilot spectrum

The UV spectra of the isolated compound showed wavelength of maximum absorbance (λ_{max}) at 265 and 298 in ethyl acetate and methanol, respectively [Table 2].

Thin-layer chromatography chromatogram

Thin-layer chromatography chromatogram of extract and isolated compound

The TLC chromatogram of the extract and the isolated compound are shown in Figure 4, and the R_f value of the isolated compound was found to be 0.6. In addition, the two-dimensional-TLC chromatogram of the isolated compound is shown in Figure 5.

Structure and molecular formula of isolated compound

The molecular formula of the isolated compound is $C_{20}H_{24}N_2O_2$ which further confirmed it as Quinine (yield = 0.147%), i.e., 6-methoxyquinolin-4-yl-8-vinylquinuclidin-2-yl-methanol, through the physicochemical and spectral data available in the literature. The structure of the stated compound is given in Figure 6.

DISCUSSION AND CONCLUSION

Quinine is one of the oldest alkaloids and is mainly found in the bark of *Cinchona* genus.[18] It is chiefly used in the treatment of malaria caused by *Plasmodium falciparum* that is resistant to other antimalarials. Quinine is also used as antibacterial, antiseptic, antipyretic, mild oxytocic, local anesthetic, cardiovascular protectant, and analgesic, etc. Quinine is used in lotions and in tonic beverages that are mixed with alcohols for bitter taste.[15,19] Because of broad spectrum usage, it is a very valuable compound that has been isolated from *P. verticillatum*. It is a rare plant and comes under the category of endangered plants, and there

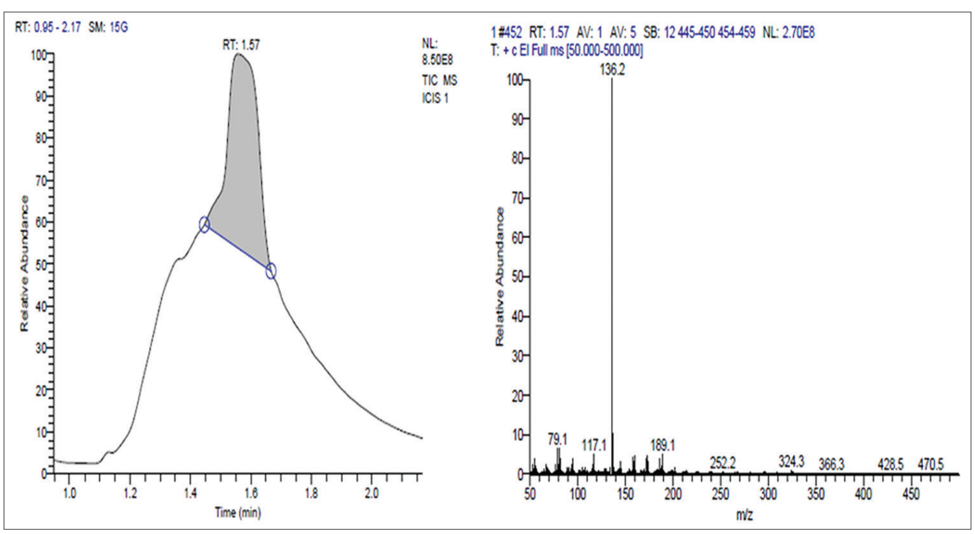

Figure 3: Mass spectrum of the isolated compound

Table 2: Wavelength of maximum absorbance of isolated compound

Threshold potential	UV spectra in ethyl acetate		UV spectra in methanol	
	Wavelength	Absorbance	Wavelength	Absorbance
0.1	1081.0	−0.047	-	-
1	601.0	−0.06	584	−0.035
5	320.8	−0.0475	445	-
10	265	−0.793	296	−0.795
15	265	−0.793	296	−0.793
20	265	−0.793	296	-
25	265	−0.793	265	-
30	265	−0.793	296	-
35	265	−0.793	-	-
40	265	−0.793	-	-
45	265	−0.793	-	-
50	265	−0.793	-	-
55	265	−0.793	-	-
60	265	−0.793	-	-
90	265	−0.793	-	-

UV: Ultraviolet

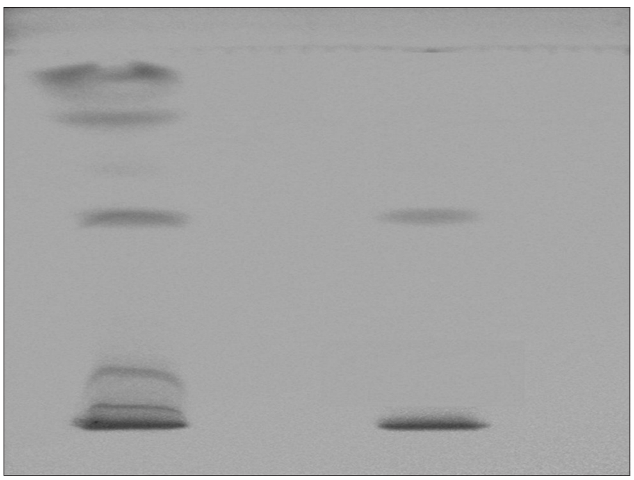

Figure 4: Thin-layer chromatography chromatogram of the extract and isolated compound

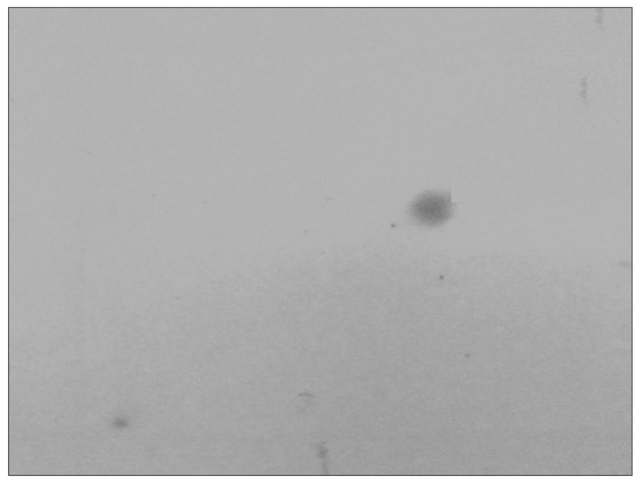

Figure 5: 2-D TLC of isolated compound

is always a possibility of adulteration in the plant material. The identification of substitution/adulteration on the basis of therapeutically active ingredients such as Quinine as the marker shall be very useful. Till date, Quinine is the identification parameter of *Cinchona* bark and this study establishes it to be used for *P. verticillatum*. The market price of Quinine is very high, and it will be difficult for commercial organizations to substitute the Mahameda plant with Quinine, just to claim the presence of Mahameda. However, if equivalent amount of Mahameda is added, it will be cheaper for the industry, in addition to its original status as a drug. Thus, Quinine can be an excellent marker for the identification of substitution/adulteration in *P. verticillatum*.

In the present study, the Quinine was isolated from rhizomes of *P. verticillatum*. The compound was confirmed by chemical tests, melting point, UV, IR, NMR, and mass spectroscopy. To the best of our knowledge, this is first report on the isolation of Quinine from *P. verticillatum*. It is further concluded that it can also be used as a supplement marker for *P. verticillatum* as the cost of the isolated compound is very high to be used as a substituent/adulterant in the *P. verticillatum* plant material. This shall provide an effective means for the standardization of the plant *P. verticillatum*.

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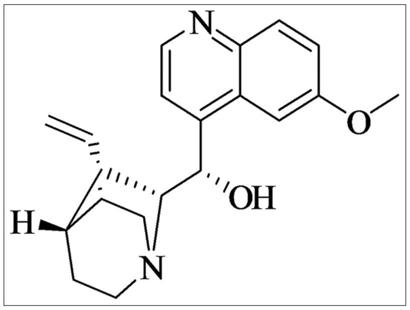

Figure 6: Structure of the isolated compound

Conflicts of interest

There are no conflicts of interest.

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