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Original Article

Corilagin: First Time Isolation from the Whole Plant of *Phyllanthus maderaspatensis* L.

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ABSTRACT

Introduction: Corilagin, an ellagitannin (chemical name β-1-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-d-glucose), was previously reported from other species of *Phyllanthus* except *Phyllanthus maderaspatensis* L. Therefore, the aim of the study was to isolate corilagin from *P. maderaspatensis* and report it as chemo-marker. **Methods:** The whole plant of *P. maderaspatensis* was subjected to hot extraction and partitioning, followed by repeated column chromatography (silica, Diaion HP-20 and Prep HPLC). The observed LC-MS/MS and NMR data were compared with reported literature to verify the structure of the isolated molecule as corilagin. **Results:** The LC-MS/MS chromatogram showed a peak at retention time 22.05 min, with *m/z* 633 for $[M - H]^-$ along with characteristic major fragments at *m/z* 463 [M - 170 - H]$^-$ and *m/z* 301 [M - 332 - H]$^-$. The NMR results were also in accordance with literature. **Conclusion:** This is the first report on isolation of corilagin from *P. maderaspatensis* whole plant for standardization and quality control of the plant.

Key words: *Phyllanthus maderaspatensis*, Corilagin, Ellagitannin, ^{1}H NMR and ^{13}C NMR.

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INTRODUCTION

Phyllanthus maderaspatensis is commonly known as Madaraas Nelli in Kannada and Hajarmani in Hindi. An alcoholic extract of the whole plant of this dominant weed plant is reported to have hepatoprotective activity in indigenous systems of medicine and is considered to be bitter, astringent, stomachic, di-uretic, febrifuge, de-obstruent and antiseptic.[1-3]

Preliminary phytochemical analysis of The *Phyllanthus* genus revealed the presence of secondary metabolites including alkaloids, flavonoids, glycosides, lignins, tannin, ellagilannins, phenols, phenyl propanoids and terpenes in the leaf, stem and roots of the plant.[4]

Corilagin (Figure 1), an ellagitannin (β-1-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-d-glucose), reported for anti-inflammatory and hepatoprotective activity. Previously it has been isolated from several plants, including several species of *Phyllanthus*. Presence of corilagin is responsible for activity of the herb therefore used as a quality control marker.[5] However, the present study aimed to isolate it from *P. maderaspatensis* whole plant. Therefore, this is the first report on isolation of corilagin from *P. maderaspatensis*.

MATERIALS AND METHODS

Instruments

VNMRS-400 "Agilent-NMR" (Fitchburg, Massachusetts, US) was used for NMR acquisition and API 2000 (Applied Biosystems/MDS SCIEX, Canada) mass spectrometer coupled with Electron Spray Ionization (ESI) was used for LC-MS analysis. Silica gel (#60-120) and Diaion HP20 was used for column chromatography. Thin layer chromatography (TLC) was performed using precoated silica gel 60 F_{254} and Shimadzu semi-preparative HPLC LC-8A was used for further purification. All LR grade chemicals and reagents were purchased from LOBA chemicals Mumbai.

Plant Material

The whole plant of *P. maderaspatensis* was collected from Krishnagiri, Tamilnadu, India, in January 2017 and was authenticated by the botanist Dr. Kannan R. A voucher specimen (PM-001) has been deposited at the Pharmacognosy Division, The Himalaya Drug Company, Bengaluru, Karnataka, India.

Extraction and isolation

Shade dried material (*P. maderaspatensis* whole plant) was purchased. Same material was pulverized using pulverizer (8.0 mm) and extracted with Methanol (MeOH): Water (2:1) followed by concentration to obtain 200g of crude extract. The extract was suspended in 500 ml water by means of heating, sonication and stirring. Afterwards, liquid-liquid partitioning was performed with Ethyl acetate (EtOAc) (1:1) and the process was repeated four times to get 46g of EtOAc fraction and 150g of water fraction. The water fraction was again fractionated with *n*-butanol (1:1) for three times to get 32g of *n*-butanol fraction. Based on HPLC results comparision (Figure S2), the butanol fraction was chosen for further separation. Diaion HP20 was chosen for column chromatography and packed in a column in a ratio 1:7 (Charged material: stationary phase). The elution was started with H_2O followed by increasing percentage of MeOH. The corilagin enriched fraction was eluted with 30% of MeOH in H_2O. The same selected for preparative HPLC for further purification. Purification was performed in isocratic mode using 25% of MeOH in H_2O (HPLC-grade) mobile phase and Kromasil 100-7-C_{18} (21.2 X 250 mm) semi preparative HPLC column. The flow rate was 15 mL/min with 4.0 mL of injection volume and 25.0 mg/mL of sample concentration. The major peak in semi preparative HPLC was collected, dried under vacuum and analyzed further for purification determination and spectroscopic analysis. The detailed system for analytical HPLC mentioned in supplementary information Table S1. HPLC chromatogram and UV comparison is presented in Figure S1-S3. Spectroscopic data for corilagin presented in Table 1.

RESULTS AND DISCUSSION

The hydro-alcoholic extract of *P. maderaspatensis* on repeated column chromatography yielded corilagin (Figure 1). The HPLC was showing

Table 1: ^{1}H and ^{13}C NMR data of compound 1 in CD_3COCD_3.

Position	δ_H (*J* in Hz)[a]	δ_C [b]
$C_{4'''}$	4.45 (1H, br s, $C_{4'''}H$)	61.3
$C_{6'''}$	4.51 (1H, t, $C_{6'''}H_b$, *J*=8 Hz)	63.4
	4.09 (1H, dd, $C_{6'''}H_a$, *J*=11.2, 8.5 Hz)	
$C_{2'''}$	4.06 (1H, br s, $C_{2'''}H$)	68.1
$C_{3'''}$	4.82 (1H, br s, $C_{3'''}H$)	69.7
$C_{5'''}$	4.93 (1H, t, $C_{5'''}H$, *J*=11.2 Hz)	74.8
$C_{1'''}$	6.36 (1H, d, $C_{1'''}H$, *J*=2 Hz)	93.3
C_3	6.68 (1H, s, C_3H)	107.3
$C_{3'}$	6.83 (1H, s, $C_{3'}H$)	109.1
$C_{2''}$, $C_{6''}$	7.12 (2H, br s, $C_{2''}H$, $C_{6''}H$)	109.2
C_1	-	109.9
$C_{1'}$	-	115.0
$C_{1''}$	-	115.6
C_2	-	119.9
$C_{2'}$	-	124.7
C_5	-	124.9
$C_{5'}$	-	136.3
$C_{4''}$	-	138.2
C_6	-	138.4
C_4	-	143.9
$C_{6'}$	-	144.0
$C_{4'}$	-	144.5
$C_{3''}$, $C_{5''}$	-	144.9
$C_{7''}$	-	164.2
$C_{7'}$	-	166.3
C_7	-	167.7

a) 400 MHz, b) 100 MHz.

0.45% of its content in extract. It was a pale-yellow powder with melting point of 210°C. Its molecular formula was established as $C_{27}H_{22}O_{18}$ by comparing LC-ESI-MS/MS fragmentation pattern with reported literature.[6] The LC-MS chromatogram (Figure S4) of this compound showed peak retention time 22.05 min with *m/z* 633 for $[M - H]^-$. Further, MS/MS data showed fragment ions at *m/z* 463 $[M - 170 - H]^-$ (loss of galloyl acid) and *m/z* 301 $[M - 332 - H]^-$ (loss of galloylglucose) which are characteristic of the fragmentation pattern of ellagitannins and tentatively confirm this compound as ellagitannin.[6] Further characterization was done with the help of NMR data. ^{1}H NMR (Table 1) (Figure S5 and S6) showed the presence of four aromatic protons at δ_H 6.68 (1H, s, C_3H), 6.83 (1H, s, $C_{3'}H$), 7.12 (2H, br s, $C_{2''}H$ and $C_{6''}H$) respectively. Broad singlets at δ_H 4.06, δ_H 4.82 and δ_H 4.45 were observed due to protons present at $C_{2'''}$, $C_{3'''}$ and $C_{4'''}$ of glucose moiety, respectively. The $C_{1'''}$ proton of the glucose moiety appears as a doublet at δ_H 6.38 with *J*=2 Hz. The proton at $C_{6'''a}$ appears as doublet of doublet at δ_H 4.09 with *J*=11.2 and 8.5 Hz. However, the proton at $C_{6'''b}$ appears as triplet at δ_H 4.51 with *J*=8.0 Hz. The proton present at $C_{5'''}$ was also observed as a triplet at δ_H 5.00 with *J*=11.2 Hz. In the ^{13}C NMR spectra (Table 1) the carbons of phenyl rings and galloyl moiety at position C_3, $C_{3'}$, $C_{2''}$ and $C_{6''}$ appears at δ_C 107.3, 109.1 and 109.2 respectively. Furthermore, the δ_C values at 164.2, 166.3 and 167.7 revealed the presence of carbonyl carbons at C_7, $C_{7'}$ and $C_{7''}$ respectively.

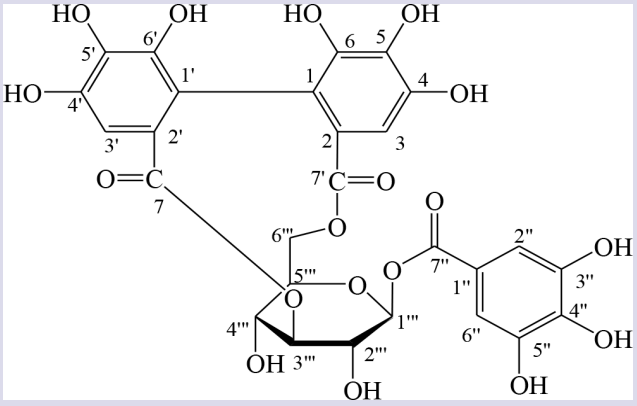

Figure 1: Chemical structure of compound corilagin.

All the spectroscopic data were in accordance with the reported literature[7] and on that basis, this compound was confirmed as corilagin.

CONCLUSION

Ellagitannins are reported from various *Phyllanthus* species as chemomarkers. However, to the best of our knowledge, this communication is the first report on isolation of corilagin from *P. maderaspatensis* whole plant which can be further used for quality control of plant.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

ABBREVIATIONS

HPLC: High performance liquid chromatography; **LC-MS:** Liquid chromatography–mass spectrometry; **LC-MS/MS:** Liquid Chromatography with tandem mass spectrometry; **ESI:** Electrospray ionization; **NMR:** Nuclear Magnetic Resonance; **MeOH:** Methanol; **EtOAc:** Ethyl acetate; **CD_3COCD_3:** Deuterated acetone; **H_2O:** Water; **TLC:** Thin Layer Chromatography.

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Supplementary data

^{1}H NMR, ^{13}C NMR and ESI-MS spectra and HPLC chromatogram can be found in online version.

Supplementary Data

1) Table S1: Chromatographic conditions.

Instrument	:	**Shimadzu HPLC; Prominence-I LC-2030 with UV detector with Class LC solution software**		
Column	:	Phenomenex Luna, C_{18}, 5µ (250 x 4.6 mm), reverse phase.		
Detection	:	UV Detector at 254 nm		
Gradient conditions		**Time (min)**	**Solvent A Concentration**	**Solvent B Concentration**
		0.01	90	10
		5.00	85	15
		12.00	80	20
		20.00	75	25
		28.00	40	60
		30.00	90	10
		35.01	Stop	
Flow rate	:	1.5 mL/min		

2) HPLC chromatogram

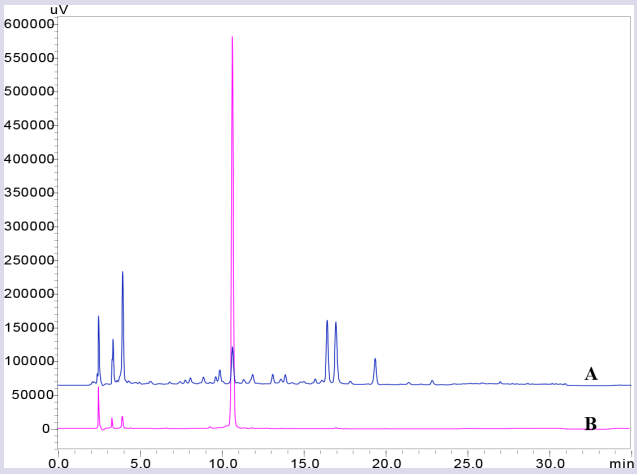


Figure S1: HPLC chromatogram of *Phyllanthus madarspatensis* extract. A. Test solution; B. Corilagin standard.

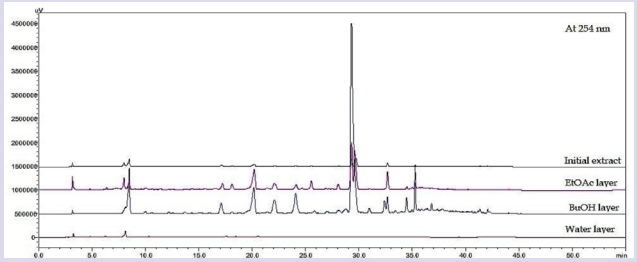
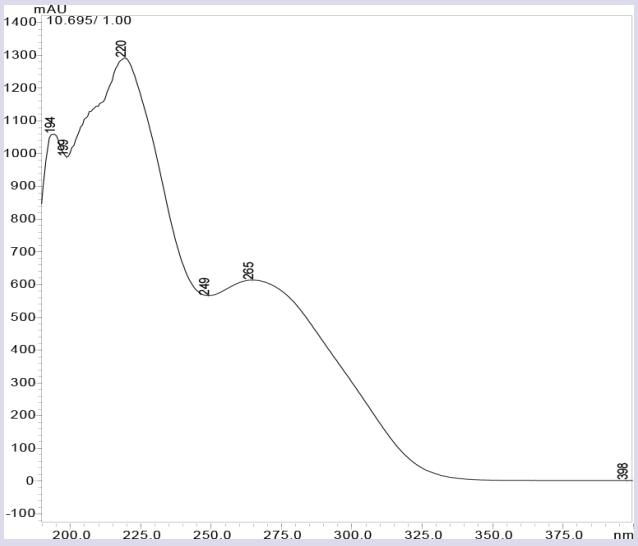


Figure S2: Comparision of HPLC chromatogram of *Phyllanthus madarspatensis* extract and partitioned fraction (Water, ethylacetate and butanol).

a). *Phyllanthus madarspatensis* **hydroalcholoic extract**



b). Corilagin standard

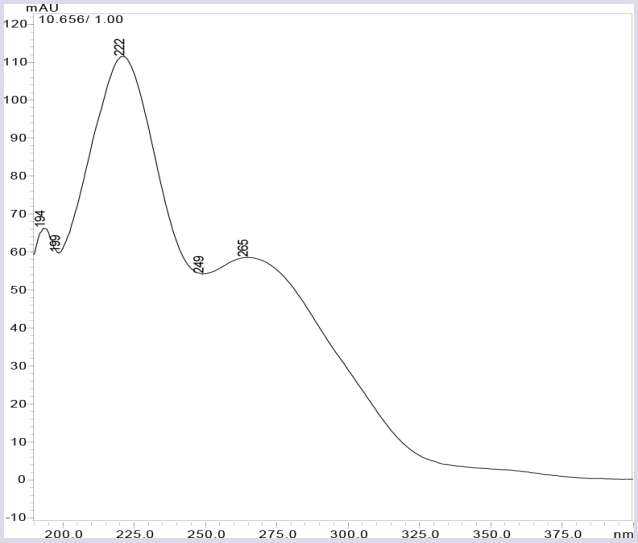


Figure S3: UV comparison of corilagin in (a) extract and (b) standard isolated.

3) LC-MS chromatogram

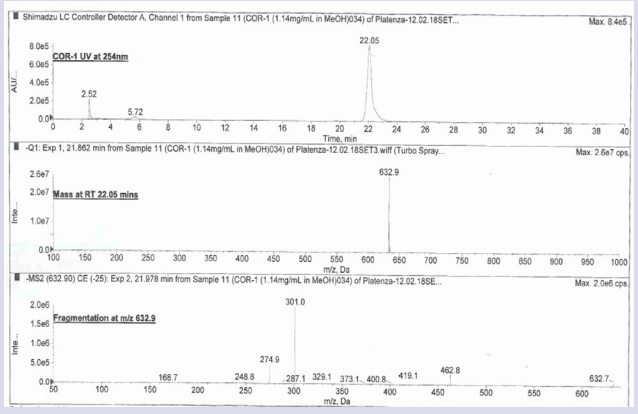


Figure S4: LC-MS spectrum of isolated compound 1 (corilagin).

4) NMR spectrum of isolated compound 1 (Corilagin)

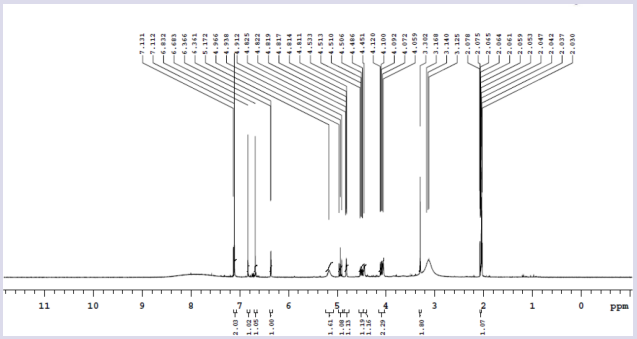


Figure S5: ^{1}H NMR spectrum of isolated compound 1 (Corilagin).

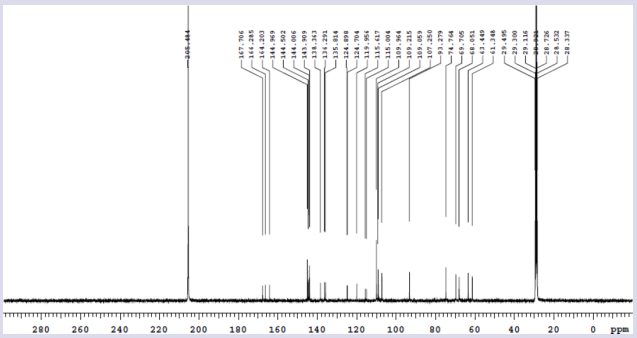
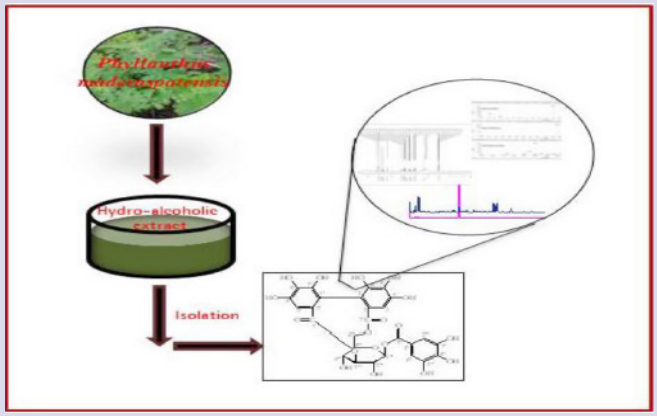


Figure S6: ^{13}C NMR spectrum of isolated compound 1 (Corilagin).

PICTORIAL ABSTRACT



SUMMARY

1. First report on isolation of corilagin from *Phyllanthus maderapatensis* L.
2. Characterization of corilagin using LC-MS/MS and NMR
3. HPLC analysis of corilagin

ABOUT AUTHORS

Mr. Abhilash K is Senior Research Associate in Phytochemistry Department of Himalaya Drug Company Bangalore. Previously, he was Senior Research Officer at Phytochemistry Division Natural Remedies Pvt Ltd, Bangalore. He has done Master of Science from Alagappa University, Karaikudi . He has around 12 years' experience in the field of Natural Product Research. During his career he worked for organizations such as USP, ICMR, Merck, Chromadex, Phytolab GmbH etc for isolation of phytocompound reference standards and monograph preparations.

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Mr. Sharath Kumar L M is working as a Research Scientist at Phytochemistry-R&D in the Himalaya Drug Co., R&D. He is having total 14 year of research experience in the field of isolation, characterization, method development and validation by HPLC and LC-MS/MS in the field of natural products. He is leading a bio-analytical team working on a projects like Pharmacokinetic studies of herbal actives, drug inhibition studies (in-vitro assay). Also, he is involved in the development of herbal actives for pharma and personal care products.

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Dr. Babu is Ph.D in Organic Chemistry and has more than 20 years of experience in natural products chemistry, drug discovery, new product development and biotechnological aspects of medicinal plants. He has extensively worked on marine fauna during doctoral studies and identified sulfated sphingolipids for the first time. He is connected with farmers and herb collectors and promoting good agricultural and collection practices and guided many students to take up this fascinating research as career option.

Dr. Ganesh Muguli is Principal Scientist in Phytochemistry Department of Himalaya Drug Company Bangalore. Previously, he was Assistant Manager in Analytical Method Development at J B Chemicals and Pharmaceuticals Ltd at Mumbai. He has done Ph.D from JSS University Mysore. He has around 18 years' experience in the field of Natural Product Research. During his career he worked on new method development for finished formulations, worked for USFD and TGA approvals for formulations.