

Research Article

Isolation of Novel Phytoconstituents from the Bark of Wonder Tree: *Prosopis Cineraria* (L.) Druce

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Abstract: A phytochemical investigation was conducted on the bark of *Prosopis cineraria* (L.) Druce (subfamily Mimosaceae, family Leguminosae/Fabaceae). Two Phytoconstituents from bark namely paeonol and ferulic acid were isolated from the ethanol extract of air dried bark of *Prosopis cineraria* by column chromatography, analysed and identified by its mass fragmentation pattern and spectrophotometric data. Their structures were established on the basis of extensive spectroscopic (IR, MS, NMR) data analysis and by comparison of the data obtained with those of the literature.

Keywords: *Prosopis cineraria* (L.) Druce, Paeonol, Ferulic acid, NMR

INTRODUCTION

Prosopis cineraria (L.) Druce belongs to a cosmopolitan genus *Prosopis* the sub family Mimosaceae, family Leguminosae/ Fabaceae [1]. The genus *Prosopis* comprises about 44 species of trees and shrubs [2], and it is an important member of arid, semiarid environments of tropical and sub-tropical region around the world [3]. Most species of *Prosopis* originate in the Americas, and only four species of *Prosopis* is occurring outside the America, in region like Africa and Southwest Asia [4, 5]. In India, *P. cineraria* and *P. chilensis* are found wildly. All Closely related species of *Prosopis* have similar morphology, and identification can be difficult, especially in the absence of flowers and fruit. In addition, the environment can have considerable effects on plant form, leaf characteristics, and growth [6], and closely related species of *Prosopis* are known to hybridize readily [7], the taxonomy of the genus *Prosopis* is complicated and the classification has been re-organized a number of times since the genus was first described [8]. *Prosopis cineraria* (L.) Druce is a multipurpose tree species. It holds an important place in the rural economy in a north-west region of Indian subcontinent [2]. It is the golden tree of Indian deserts, plays a vital role in preserving the ecosystem of arid and semi-arid areas. Since all the parts of the tree are useful, it is called kalp taru. It is also known as the 'wonder tree' and the 'king of desert' [8-11].

The conservation of this tree is a religious tenet of Rajasthan's Bishnoi community in India. The Government of India has also instituted the 'Amria Devi Bishnoi National Award for Wildlife Conservation' in the memory of Amrita Devi Bishnoi, who in 1731 sacrificed her life to protect these trees in Khejarali village near Jodhpur [12]. The Khejri tree is appropriately being used as a theme for the stamp (Plate

4.2) released on 5th June, 1988, World Environment Day, by the Department of Indian Posts. The Indian Government security press has released 15,00, 000 number of printed stamps [13].

Phytoconstituents

A variety of phytoconstituents have been isolated from different parts of *Prosopis cineraria* (L.) Druce. Flowers contain patuletin glycoside patulitrin [14], sitosterol, spicigerine, Flavone derivatives Prosogerin-A and Prosogerin B [15]. Leaves contain steroids like campesterol, cholestrol, sitosterol and stigmasterol, actacosanol, hentriacontane, methyl docosanoate, Diisopropyl-10, 11-dihydroxyicosane-1,20-dioate, Tricosan-1-ol, and 7,24-Tirucalladien-3-one along with a piperidine alkaloid spicigerine [16]. Seeds contain Prosogerin C [17], Prosogerin D [18], Prosogerin E, Gallic acid, patuletin, patulitrin, luteolin, and rutin [19]. The seed lipids contain a relatively large proportion of unsaturated fatty acids, with linoleic and oleic acids being predominant [20]. Dried pods contain 3-benzyl-2-hydroxy-urs-12-en-28-oic acid, maslinic acid 3-glucoside, linoleic acid, prosphylline, 5,5'-oxybis- 1,3-benzendiol, 3,4,5, trihydroxycinnamic acid 2- hydroxy ethyl ester and 5,3',4'-trihydroxyflavanone 7- glycoside [21] and the whole plant contains methyl heptacosanoate, heneicosanoic acid, 4-hydroxy benzoic acid, methyl 4- hydroxycinnamate, methyl 2-methoxy-5-hydroxycinnamate and O-Coumaroylglycol [22].

In this research paper, we report the isolation and characterization of paeonol (VND-1) and ferulic acid (VND-6) from bark of *Prosopis cineraria* (L.) Druce. These compounds are reported for the first time from this plant.

MATERIALS AND METHODS

Plant Material

The plant bark of *Prosopis cineraria* (L.) Druce was collected in the months of April 2008, from Village Pali, District Mahendergarh, Haryana, India and was identified and authenticated by Dr. H. B. Singh Head, Raw Material, herbarium and museum division, NISCAIR, New Delhi Ref.Niscair/Rhmd/Consult/-2008-09/971/02) and a sample has been retained in the department for future reference.

Chemicals and Solvents

All reagents used were either analytical grade or chromatographic grade were purchased from Sigma-Aldrich and Merck-India.

Extraction and Isolation

The bark of *Prosopis cineraria* (L.) Druce was air dried in shade and then grounded with a mechanical grinder. 1 kg of the air dried bark powder of *Prosopis cineraria* (PC) was extracted using 95% ethanol using soxhlet apparatus for 72 h. The solvent was evaporated under vacuum using rotatory flash evaporator. The yield of the ethanol extract was 18.28% w/w and it was used for isolation of ferulic acid. 60 gram of PC extract was poured on the top of the column packed with silica gel. Elution mixture of isoamyl alcohol: acetic acid: water (1:1:2) to afford a light yellow colour solid compound (1.34 g). It was recrystallized from hexane to yield 0.68 g of vnd-6. Thin layer chromatography of PC extract in isoamyl alcohol: acetic acid: water (1:1:2) showed the presence of yellowish green coloured single spot. (Rf 0.89). The remaining rhizomes powder was soaked in ethanol for another six days. The solution was filtered, and the combined filtrates were concentrated using a rotary evaporator to yield 60.3 g of a brown-oily bark ethanol extract.

This ethanol extract (60.3 g) underwent silica gel flash column chromatography (Merck 1.07747) using 10% polarity increments from 90: 10, chloroform: ethanol to 100% ethanol whereby 100 ml fractions were collected. The flash column chromatography fractions 5, 6 and 7 were combined (2.7 g) and subjected to radial chromatography to produce five fractions. The combined fractions 1, 2 and 3 were fractionated over silica gel (Merck 1.07749) on preparative thin layer chromatography to give 64 mg of vnd-1.

Equipment Used for Structural Elucidation

Different spectroscopic methods were used to elucidate the structure of isolated compound. Among the spectroscopic techniques IR, ¹H-NMR, ¹³C-NMR and GC-MS were carried out. The infra red spectrum was recorded on FTIR Perkin Elmer, ¹H-NMR and ¹³C-NMR spectra were recorded using CDCl₃ as solvent on Bruker Advance II 400 NMR and GC-MS spectra were recorded at high resolution on a mass spectrometer (Perkin Elmer Auto system) at spectrometer SAIF Panjab University, Chandigarh, the data are given in m/z values.

Features of Spectroscopic Analyses of Fraction of *Prosopis cineraria* Bark

VND-6

Identity of the compound VND-6 was ascertained from its NMR (¹H, ¹³C and DEPT), IR and mass spectrometry as well as by comparison with data reported in the literature [23]. Its ¹H NMR spectrum displayed a singlet corresponding to the methoxy group at δ 3.98 ppm. The presence of three signals at δ 6.84 (d; J=8.2 Hz), 7.03 (dd; J=8.2 and 1.6 Hz) and 7.16 (d; J=1.6 Hz) could be attributed to the aromatic protons i.e. H-5, H-6 and H-2 respectively. The two olefinic protons in the side chain of the compound (i.e. H-7 and H-8), appeared as doublets with J= 16 Hz at δ 6.31 ppm and 7.53 ppm respectively. The two characteristic broad singlets at δ 9.39 ppm and 11.67 could be attributed to the phenolic and the carboxylic proton.

The ¹³C NMR spectrum displayed 10 signals corresponding to six aromatic carbons, one methoxyl group, two olefinic carbons and one carbonyl carbon. DEPT-135 NMR showed presence of six positive peaks, whereas the peaks corresponding to four quaternary carbons (i.e., C-1, C-3, C-4 and C-9) were absent. The data was in agreement with the proposed structure of ferulic acid. The IR spectrum of the compound exhibit peaks at 3433 cm⁻¹ (-OH str), 1678 cm⁻¹ (-C=O str), 1607 (-C=C- str) which support structure of the given compound. The ESI-MS displayed a molecular related ion [M+H]⁺ at 195 in agreement with the proposed structure Fig.2.

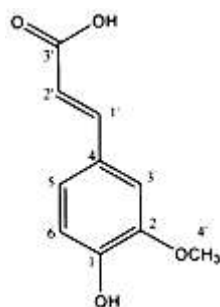
VND-1

White needles (64 mg). IR cm-1: 3432, 2925, 1720, 1383, 1246, 1021. EIMS for C₉H₁₀O₃ m/z (rel. int.): 166 [M+] (31.3%), 151 (100%), 108 (16.5%), 95 (21.0%), 43 (34.6%). ¹H NMR (400 MHz, CDCl₃) δ : 2.56 (3H, s, CH₃), 3.84 (3H, s, OCH₃), 6.42-6.45 (2H, m, H-3, H-5), 7.63 (1H, d, J=8.8 Hz, H-6), 12.75 (1H, s, OH). ¹³C NMR (100 MHz, CDCl₃) δ : 114.1 (C-1), 165.4 (C-2), 101.0 (C-3), 166.3 (C-4), 107.9 (C-5), 132.5 (C-6), 202.8 (C=O), 55.8 (OCH₃), 26.4 (CH₃).

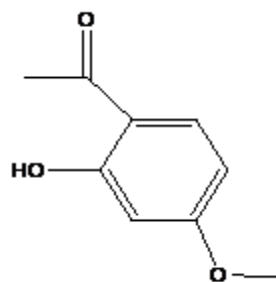
RESULTS AND DISCUSSION

Ferulic acid (vnd-6) was obtained from bark as light yellow colour solid compound The ESI-MS displayed a molecular related ion [M+H]⁺ at 195 in agreement with the proposed structure of ferulic acid. And Paeonol (vnd-1) was isolated from the barks successive ethanol extract as white needles. Mass spectrum of this compound showed a molecular ion peak at m/z 166, which is in agreement with the molecular formula C₉H₁₀O₃ of paeonol. ¹H NMR spectrum of paeonol showed that there are two singlets at δ 2.56 and 3.84 representing two methyl groups attached to respective quaternary carbonyl, and oxygen. A signal for H-3, H-5 appeared at δ 6.42-6.45 as a multiplet. Proton H-6 showed a doublet which appeared

at δ 7.63 with a coupling constant of 8.8 Hz. A hydrogen-bonded, six-membered ring phenolic proton was displayed very down-field at δ 12.75. ^{13}C NMR data of this compound showed that there were nine signals which represent nine different carbons. The signal at δ 202.8 was assigned to the carbonyl group of C-1. Moreover, signals at δ 101.0, 107.9 and 132.5 were assigned to respective aromatic carbons of C-3, C-5 and C-6. Signal at δ 165.4 was assigned to C-2 which is known as an aromatic carbon attached to a hydroxyl group whereas the appearance of the aromatic C-4 with the methoxy substitution was seen at δ 166.3 due to the electron donating feature of the oxygen atoms in those



Ferulic acid



Paeonol

CONCLUSION

The isolation and identification of paeonol and ferulic acid from the barks of *Prosopis cineraria* (L.) Druce was the first ever to be reported from this plant. The work was carried out by utilizing several kinds of chromatographic separation techniques and spectroscopic analyses.

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