Abstract

Adenanthera pavonina Linn and Erythrina variegata Lam are being used in the Indian traditional systems of medicine for the cure of variety of ailments. Present study was designed to isolate and characterize phytoconstituents from the leaves of A. pavonina Linn and E. variegata Lam. Methanolic extracts of the leaves of A. pavonina and E. variegata were prepared and slurry on silica gel was prepared from these extracts separately. Silica gel was packed in chromatography column and dried slurry was loaded. The column was eluted successively with different solvents in the order of increasing polarity. Chromatographically identical fractions were combined together and concentrated. Isolated phytoconstituents were crystallized and characterized by UV, IR, 1H-NMR, 13C-NMR and MS spectroscopy. Isolated phytoconstituents 1, 2, 3 and 4 were characterized as n-tricosanol, α-D-glucopyranosyl-(2→1')-α-D-glucopyranosyl-(6'→2')-α-D-glucopyranosyl-(6→1' 2→2)-α-D-glucopyranoside, hexatetracontan-1-ol and n-octanyl-1β-D-glucopyranosyl-(6"→1')-β-D-glucopyranoside respectively. From the present study, it can be concluded that the existing knowledge concerning the phytoconstituents from the leaves of A. pavonina and E. variegata may be enlarged by the current phytoconstituents investigation which is valuable as these drugs are being used in the Indian traditional systems of medicine.

Keywords: Adenanthera pavonina, Erythrina variegata, isolation, phytoconstituent, column chromatography

Introduction

Adenanthera pavonina Linn belonging to the family Fabaceae is normally known as red wood (1). Traditionally, its ground seed is used for the treatment of diverse ailments such as inflammation, arthritis, rheumatism, convulsion, epilepsy, paralysis, spasm, cholera, boils, blood disorders, hepatotoxicity and indigestion (2, 3). Scientific investigation on A. pavonina showed that the plant extracts have anti-inflammatory, analgesic, antifungal, anti-oxidant, anti-hyperlipidemic, anti-diabetic, cytotoxic and blood pressure lowering effects (4, 5, 6, 7). Its seed contains O-acetylethanolamine; leaves stigmasterol, dulcitol, octacosanol, glucosides of α-sitosterol; bark stigmasterol glucoside; and pods steroids, saponins and glycosides (8, 9, 10). Pavonin, a phytoconstituent with lactone ring and exo-cyclic double bond was also isolated from its methanol soluble part (11).

The plant Erythrina variegata Lam belonging to the family Papilionaceae is normally known as Indian coral tree in English and Mandara in Hindi. Traditionally, the leaves are used as diuretic, laxative, galactagogue, emmenagogue, antihelminthic and in the treatment of joint pain (12, 13, 14). Scientific investigation on E. variegata showed that the...
plant extracts have central nervous system effect, anti-osteoporotic, cardiovascular, cytotoxic, antiulcer, anthelmintic, diuretic, analgesic and antioxidant activities (15, 16, 17, 18). The plant contains sterols like campesterol, β-amyrin, β-sitosterol; alkaloids like N-norprotosinomenine, erysodienone, protosinomenine, 3-erythroidine, erythraline, erysopine, erythramine, erysotrine, erysodine, erythratine, hyparphorine, N,N-dimethyltryptophan; isoflavones like indicanines D and E; flavonoids like genkwanin, apigenin, isovitexin, saponarin, swertisin, 5-O-glucosylisoswertisin, 5-O-glucosylswertisin; and a triterpene betulin (19, 20, 21).

Present study was carried out to explore the plants *A. pavonina* and *E. variegata* with regard to their phytoconstituents and also to enlarge the existing knowledge concerning their phytoconstituents. Hence, present study describes the isolation and characterization of phytoconstituents from the leaves of *A. pavonina* and *E. variegata*.

### Materials and Methods

**Collection and authentication of plant specimens:** Fresh leaves of the plants *Adenanthera pavonina* Linn and *Erythrina variegata* Lam were collected from Pallavaram, Chennai, Tamilnadu and authenticated by Plant Anatomy Research Center, National Institute of Herbal Science, Chennai (ref. no.: PARC/2011/954 and PARC/2011/955).

**Chemicals and instruments:** All the solvents and chemicals used in the study were of analytical grade. Petroleum ether 60-80°C, methanol, formic acid, toluene, and diethyl ether were obtained from SD Fine Chem Pvt Ltd, Mumbai. Ethanol, Chloroform, Silica gel 60-120 mesh size, and Silica gels G were obtained from Merck Ltd, Mumbai. Melting point apparatus (Remi Equipments, India), Electronic balance (Sartorious, India), UV Spectrophotometer (160A UV-Vis, Shimadzu, Japan), FTIR Spectrophotometer (Bio-Red).

**Preparation of extracts:** Collected leaves of the plants *A. pavonina* Linn and *E. variegata* Lam were washed with distilled water, dried under shade at room temperature and powdered to a coarse powder (500 g). The powder was packed in muslin cloth and subjected to Soxhlet extraction with methanol for 72 h at 50°C. Methanolic extracts were filtered through Whatmann No.1 filter paper and filtrates were concentrated to dryness under reduced pressure and temperature in Rotavapor. Extracts were stored in freezer and used for phytochemical isolation. Procedure was followed separately for both the plants (22).

**Preparation of slurry for column chromatography of the extracts:** *A. pavonina* (10 g) and *E. variegata* (10 g) extracts were used for the preparation of slurry. The concentrated extract of the plant material was taken in a China dish and heated continuously on a water bath by gradual addition of methanol in small portions with constant stirring till desired consistency was obtained. A weighed quantity of silica gel for column chromatography was then added slowly with continuous mixing with a steel spatula until the whole methanolic solution of the extract adsorbed on silica gel particles. It was dried in air and the larger lumps were broken by rubbing between hands and finally passed through a sieve No 8 to get uniform particle size.

**Packing of column and isolation of phytoconstituents:** A column of 3 ft height and 16 mm internal diameter was taken, cleaned properly and dried. The lower end of the column was plugged with non-absorbent cotton wool. The column was clamped and fitted in vertical position on a stand. The column was then half filled with petroleum ether 60-80°C. Silica gel for column (60-120 mesh size) was then poured in small portions and allowed to settle down and the dried slurry was loaded over the column and then eluted successively with different solvents in the order of increasing polarity. The developments and elution of the column were carried out with successive series of different solvents in various combinations, such as petroleum ether (100%), petroleum ether:chloroform (75:25, 50:50, 25:75), chloroform (100%), chloroform:methanol (99:1,
98:2, 97:3, 95:5, 90:10, 80:20) and methanol (100%) to isolate the phytoconstituents.

**Homogeneity of the fractions:** The fractions collected were subjected to thin layer chromatography (TLC) to check homogeneity of various fractions. Chromatographically identical fractions (having same R values) were combined together and concentrated. Isolated phytoconstituents were crystallized with suitable solvent system.

**Spectral characterization of isolated phytoconstituents:** Ultraviolet (UV) spectra of isolated phytoconstituent were recorded on UV spectrophotometer in methanol at Faculty of Pharmacy, Integral University, Lucknow. Infrared (IR) spectra were recorded on FTIR spectrophotometer using KBr pellets. 1H-NMR spectra were screened on Bruker spectrospin 400 MHz instrument using deutarated chloroform (CDCl₃) as solvent and tetramethylsilane (TMS) as internal standard. 13C-NMR spectra were recorded on Bruker Spectrospin 400 MHz in 5 mm spinning tubes at 27°C. Mass spectra (MS) were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 instruments equipped with direct inlet probe system. IR, 1H-NMR, 13C-NMR and MS spectral characterization were carried out at SAIF, Central Drug Research Institute (CDRI), Lucknow. TLC spots were visualized by exposure to iodine vapors and UV radiation.

**Results**

Structures of the phytoconstituents 1, 2 isolated from methanolic extract of the leaves of *A. pavonina* and phytoconstituents 3, 4 isolated from methanolic extract of the leaves of *E. variegata* are shown in figure 1.

**Spectral characterization of isolated phytoconstituents from Adenanthera pavonina:** Elution of the column with chloroform:methanol (9:1) yielded yellowish mass of phytoconstituent (1) (110 mg). R value [Solvent system: chloroform:methanol (9:1): 0.46; mp: 120-125°C; UV \( \lambda_{max} \) (MeOH): 214 nm; IR \( \nu_{max} \) (KBr): 3401, 2925, 2841, 1647, 1216, 1096, 770 cm⁻¹; 1H-NMR (CDCl₃): \( \delta \) 3.37 (2H, t, \( J= 6.0 \) Hz, H-1), 2.30, (2H, m, H-2), 2.02 (2H, m, CH₂), 1.72 (2H, m, CH₂), 1.58 (4H, m, 2xCH₂), 1.41 (4H, brs, 2xCH₂), 1.29 (6H, brs, 3xCH₃), 1.25 (2H, brs, 11xCH₂), 0.87 (3H, t, \( J=6.9 \) Hz, Me-23); 13C-NMR (CDCl₃): \( \delta \) 62.95 (C-1), 32.94 (CH₂), 31.84 (CH₂), 29.90 (16xCH₂), 29.57 (CH₂), 29.37 (CH₂), 22.90 (CH₂), 14.32 (C-12), 75.08 (C-22), 69.84 (C-32), 73.97 (C-42), 64.51 (C-52), 13.18 (C-12). 22.03 (Me-23); ESI-MS 

Elution of the column with chloroform: methanol (9:1) furnished colourless crystals of phytoconstituent (2) (105 mg). R value [Solvent system: chloroform:methanol (9:1): 0.49; mp: 130-132°C; UV \( \lambda_{max} \) (MeOH): 264 nm; IR \( \nu_{max} \) (KBr): 3401, 3345, 3215, 3019, 2951, 2842, 1647, 1384, 1215, 1095, 759 cm⁻¹; 1H-NMR (CDCl₃): \( \delta \) 5.12 (1H, d, \( J=3.9 \) Hz, H-1α), 4.49 (1H, d, \( J=6.1 \) Hz, H-12), 4.06 (1H, d, \( J=5.1 \) Hz, H-12), 4.01 (1H, d, \( J=3.1 \) Hz, H-12), 3.95 (1H, m, H-2), 3.89 (1H, m, H-22), 3.84 (1H, m, H-5), 3.82 (1H, m, H-52), 3.80 (1H, m, H-52), 3.78 (1H, m, H-52), 3.76 (1H, m, H-22), 3.75 (1H, m, H-22), 3.72 (1H, m, H-3), 3.69 (1H, m, H-32), 3.65 (1H, m, H-32), 3.61 (1H, m, H-32), 3.59 (2H, m, H-4, H-42), 3.56 (1H, m, H-42), 3.54 (1H, m, H-42), 3.51 (2H, brs, H2-62), 3.25 (2H, d, \( J=9.8 \) Hz, H-6), 3.13 (2H, d, \( J=5.1 \) Hz, H-32), 3.11 (2H, d, \( J=4.8 \) Hz, H-22); 13C-NMR (CDCl₃): \( \delta \) 103.27 (C-1), 84.98 (C-2), 73.08 (C-3), 71.83 (C-4), 78.19 (C-5), 60.89 (C-6), 99.32 (C-12), 83.38 (C-22), 72.66 (C-32), 71.33 (C-42), 78.10 (C-52), 62.86 (C-62), 58.29 (C-12), 76.40 (C-22), 72.10 (C-32), 69.53 (C-42), 77.69 (C-52), 64.65 (C-62), 94.05 (C-12), 76.40 (C-22), 71.94 (C-32), 65.99 (C-42), 76.90 (C-52), 62.95 (C-62), 62.95 (C-62), ESI MS (m/z, rel. int.): 666 [M]+ (C₃₂H₄₈O₂₁) (21.3), 504 (12.6) 342 (77.8), 324 (10.5), 179 (6.6).

**Spectral characterization of isolated phytoconstituents from Erythrina variegata:** Elution of the column with chloroform: methanol (4:1) furnished colourless crystals of phytoconstituent (3) (130 mg). R value [Solvent phytoconstituents from the leaves of *A. pavonina*
system: chloroform: methanol (4:1); 0.41; mp: 125-130°C; UV $\lambda_{max}$ (MeOD): 207 nm; IR $\nu_{max}$ (KBr): 3411, 2975, 2842, 2842, 1602, 1475, 1215, 1046, 928, 876, 758 cm$^{-1}$. $^1$H-NMR (MeOD): $\delta$ 3.82 (2H, t, $J$ = 9.0 Hz, H$_2$-1), 2.05 (2H, m, H$_2$-2), 1.59 (2H, m, CH$_2$), 1.46 (2H, m, CH$_2$), 1.38 (6H, brs, 3×CH$_2$), 1.29 (74H, brs, 37×CH$_2$), 0.89 (3H, t, $J$ = 6.5 Hz, Me-46); $^{13}$C-NMR (CDCl$_3$): $\delta$ 103.27 (C-1), 84.98 (C-2), 73.08 (C-3), 71.83 (C-4), 78.19 (C-5), 60.89 (C-6), 99.32 (C-12 ), 83.38 (C-22 ), 72.66 (C-32 ), 71.33 (C-42 ), 78.10 (C-52 ), 62.86 (C-62 ), 98.29 (C-12 2 ), 76.40 (C-22 2 ), 72.10 (C-32 2 ), 69.53 (C-42 2 ), 77.69 (C-52 2 ), 64.65 (C-62 2 ), 94.05 (C-12 2 2 ), 76.40 (C-22 2 2 ), 71.94 (C-32 2 2 ), 65.99 (C-42 2 2 ), 76.90 (C-52 2 2 ), 62.95 (C-62 2 2 ); ESI-MS (m/z, rel. int.): 662 [M]$^+$ (C$_{46}$H$_{94}$O) (100).

Elution of the column with chloroform: methanol (4:1) furnished colorless crystals of phytoconstituent (4) (110 mg). R$_f$ value [solvent system: chloroform:methanol (4:1); 0.54; mp: 110-115°C; UV $\bar{\epsilon}_{max}$ (MeOD): 244 nm; IR $\nu_{max}$

Mujahid et al
Phytoconstituents from the leaves of *A. pavonina*

Phytoconstituent (1) namely *n*-tricosanol was obtained as a yellowish mass from chloroform:methanol (9:1) eluent. Its IR spectrum showed absorption bands for hydroxyl groups (3401 cm⁻¹), unsaturation (1647 cm⁻¹) and long aliphatic chain (770 cm⁻¹). On the basis of mass and ¹³C-NMR spectra, the molecular ion peak of phytoconstituent 2 was determined at *m/z* 666 corresponding to the molecular formula of *α*-D-tetraglucoside (C₅ₓH₄₂O₂₁). The ¹H-NMR spectrum of phytoconstituent 2 displayed a numeric proton signals on one-proton doublets at δ 5.12 (*J* =3.9 Hz), 4.49 (*J* =6.1 Hz), 4.06 (*J* =5.1 Hz) and 4.01 (*J* =3.1 Hz) as three two-proton doublet at δ 3.25 (*J* =9.8 Hz), 3.13 (*J* =5.1 Hz) and 3.11 (*J* =4.8 Hz). The numeric single proton multiplets appeared from δ 3.95 to 3.54. The ¹³C-NMR spectrum of phytoconstituent 2 exhibited signals for four anomeric sugar carbons between δ 103.27 to 84.98 and other sugar carbons in the range of δ 73.08 to 62.95. On the basis of these spectral data analysis, structure of phytoconstituent 2 was characterized as *α*-D-glucopyranosyl-(2'12')-*α*-D-glucopyranosyl-(2'→1')-*α*-D-glucopyranosyl-(6''→1'')-*α*-D-glucopyranoside. It is a new glucosidic phytoconstituent from *A. pavonina*. The results of the study on *Adenanthera pavonina* clearly indicates that its leaves contain *n*-tricosanol and *α*-D-glucopyranosyl-(2'→1')-*α*-D-glucopyranosyl-(2''→1'''')-*α*-D-glucopyranoside which is supported by previous reports that the leaves of *A. pavonina* contain stigmasterol, dulcitol, octacosanol, glucosides of β-sitosterol (8, 9, 10). But, these isolated phytoconstituents; tricosanol and glucoside from *A. pavonina* are different from the alcohols and glucosides already reported in it.
Structural elucidation of isolated phytoconstituents from Erythrina variegata:

Phytoconstituent (3) namely hexatetracontan-1-ol was obtained as a yellowish mass from chloroform:methanol (4:1) eluent. Its IR spectrum showed absorption bands for hydroxyl groups (3411 cm⁻¹), unsaturation (1602 cm⁻¹) and long aliphatic chain (758 cm⁻¹). On the basis of mass and ¹³C-NMR spectra, the molecular ion peak of 3 was determined at m/z 662 corresponding to the molecular formula of C₄₆H₉₄O. The ¹H-NMR spectrum of 3 showed a two proton triplet at δ 3.82 (J=6.0 Hz) assigned to anomeric H 2-1 proton. It also showed the three two proton multiplet at δ 2.05 assigned to H2-2 and 1.59 and 1.46. The other sugar protons appeared between δ 1.38 and 1.29. A three-proton triplet at δ 0.89 (J=6.6 Hz) was accounted to the methyl protons. The ¹³C-NMR spectrum displayed signals for anomeric carbon at δ 62.95 (C-1), other carbons between δ 31.94 and 22.37. On the basis of these spectral data analysis, structure of phytoconstituent 3 was characterized as hexatetracontan-1-ol which is a new compound from E. variegata but known in other plants (25).

Phytoconstituent (4) namely n-octanyl-1β-D-glucopyranosyl-(6’→1”)-β-D-glucopyranoside was obtained as a colorless crystalline mass from chloroform:methanol (4:1) eluent. It gave positive test for reducing sugar and had IR absorption bands for hydroxyl group (3401, 3343 and 3019 cm⁻¹) with ester group (1645 cm⁻¹), pyranosyl (1384 cm⁻¹) and glycosidic linkage (1216 cm⁻¹). Vibration of a C=O linkage has been shown to produce an absorption band at 1384 cm⁻¹ supporting the presence of pyranosyl group in the phytoconstituent. On the basis of mass and ¹³C-NMR spectra, the molecular ion peak of phytoconstituent 4 was determined at m/z 454 corresponding to the molecular formula of β-D-glycoside (C₂₀H₃₈O₁₁). The ¹H-NMR spectrum of phytoconstituent 4 displayed a numeric proton proton signals on four one-proton doublets at δ 5.17 (J=7.8 Hz), 5.07 (J=7.8 Hz), 3.28 (J=7.5 Hz) and 3.22 (J=9.3 Hz), assigned to H-12, H-12 2, H₂-62 and H₂-62 2.

The two proton triplet at 3.34 (J=11.4) and a three triplet-proton at δ 0.89 (J=6.6 Hz) assigned to methyl proton. The numeric single proton multiplets appeared from δ 3.43 to 3.48. The ¹³C-NMR spectrum of phytoconstituent 4 exhibited signals for four anemic sugar carbons between δ 66.05 and 14.16, and other sugar carbons in the range of δ 99.32 to 60.92. On the basis of these spectral data analysis, structure of phytoconstituent 4 was characterized as n-octanyl-1β-D-glucopyranosyl-(6’→1”)-β-D-glucopyranoside. It is a new glycosidic phytoconstituent. Previous reports on E. variegata clearly indicates that the plant contains sterols like campesterol, β-amyrin, β-sitosterol; alkaloids like N-norprotosinomenine, erysodienone, protosinomenine, 3-erythroidine, erythrine, eryspine, erythramine, erysotrine, erysodine, erythratine, hyparphorine, N,N-dimethyltryptophan; isoflavones like indicanines D and E; flavonoids like genkwanin, apigenin, isovitexin, saponarin, swertisin, 5-O-glucosylswertisin, 5-O-glucosyliswertisin; and a triterpene betulin (19, 20, 21). The results of the study on E. variegata clearly indicates that its leaves contain hexatetracontan-1-ol and n-octanyl-1β-D-glucopyranosyl-(6’→1”)-β-D-glucopyranoside suggesting the presence of two new phytoconstituents in E. variegata.

Conclusion

The present work characterized four phytoconstituents isolated from the leaves of A. pavonina and E. variegata. The existing knowledge concerning their phytoconstituents may be enlarged by the current phytoconstituents investigation which is valuable as these drugs are being used in the Indian traditional systems of medicine.

Acknowledgement

The authors acknowledge the help rendered by Head, CIF, Central Drug Research Institute (CDRI) Lucknow for scanning spectra and also acknowledge the help rendered by Prof (Dr.) Mohammed Ali, Faculty of Pharmacy, Jamia Hamdard, New Delhi for the interpretation of isolated phytoconstituents.

Mujahid et al
References


