

Isolation of Phytoconstituents from the Stem Bark of *Bauhinia Variegata* Linn

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ABSTRACT

Fractionation of crude petroleum ether extract of the stem bark of *Bauhinia variegata* Linn (Caesalpiniaceae) led to the isolation of heptatricontan-12, 13-diol, friedelin, n-docosanoic acid, stigmaterol, β -sitosteryl, n-hexadecanoic acid, lupeol. Eriodictyol & Quercetin were isolated from methanolic extract. Their structures were elucidated by spectroscopic methods such as UV, IR, ¹H NMR and MASS. Eriodictyol was isolated for the first time from this plant.

Keywords: *Bauhinia Variegata*, Isolation, phytochemical screening

INTRODUCTION

Bauhinia variegata (Linn.), belonging to family caesalpiniaceae. It is known as mountain ebony in English, Kanchanar in Hindi. The plant is a medium-sized deciduous tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India.^[1] The bark is grey or brownish with longitudinal cracks and light pink inside. Leaves are 10-12.5 cm long, 1-foliolate, 2-lobed, not deeply cleft, rigidly subcoriaceous and deeply cordate. Flowers are pink coloured and sessile or born in short peduncled corymbs. Pods are 15-30 cm into 1.2-2.0 cm long, flat hard, compressed and debiscent. The seeds are 10-15 in pods. The various parts of the plants viz., leaves, flower buds, flower, stem, stem bark, seeds and roots are used in fever, as tonic, astringent, diarrhoea, dysentery, hemorrhoids, piles, edema, laxative, anthelmintic, antileprotic, in skin diseases, wound healing, antioitrogenic,

antitumor, in obesity, stomatitis, antidote for snake poisoning, dyspepsia, flatulence and as carminative.^[2] The chemical constituents isolated so far from the plant are β -sitosterol, kaempferol-3-glucoside, tannins,^[3] carbohydrates, amides, reducing sugars, vitamin C, crude protein, fibers,^[4] calcium, phosphorus,^[5] quercetin, rutin, quercitrin, apigenin, apigenin-7-O-glucoside,^[6] heptatriacontan-12, 13-diol and dotetracontan-15-en-9-ol.^[7] Despite the enormous medicinal properties mentioned in the traditional literature and a profound and systematic Phytochemical and pharmacological investigations of stem bark of *Bauhinia variegata* Linn., are not reported in the scientific arena, thus the present investigation is emphasizing on the investigation of phytochemical screening and evaluation of pharmacological prosperities of the bark.

How to cite this article: MMJ Vijay, B Eswarappa, YD Bodke, KV Jayadevaiah, HS Basavaraja; Isolation of Phytoconstituents from the Stem Bark of *Bauhinia Variegata* Linn; PharmaTutor; 2014; 2(9); 150-156

In the present work, we have isolated heptatricontan-12, 13-diol (KA 01), n-Docosanoic acid (KA 02), Hexadecanoic acid (KA 03), β -Sitosterol (KA 04), Stigmasterol (KA 05), friedelin (KA 06) & Lupeol (KA 07) from petroleum ether extract. Eriodictyol (KA 08) & Quercetin (KA 09) were obtained from ethanolic extract.

MATERIALS & METHODS

Collection and authentication of the plant:

The stem bark of *Bauhinia variegata* Linn., was collected from Chitradurga, Karnataka. It was authenticated by Prof. V.T. Hiremath, Department of Botany, SJM College of Arts, Commerce & Science, Chitradurga, Karnataka. A voucher specimen no 108-A is deposited in the herbarium of SJM College of Pharmacy, Chitradurga, Karnataka, India.

General chemicals & instrument details

AR grade solvents were purchased from Sigma–Aldrich chemicals.

UV: Shimadzu UV VIS-1700; IR: Bruker FTIR 5300; MASS-ESI.1cm⁻¹H-NMR (400 MHz): Bruker Avance II 400.

Extraction and isolation procedure

The shade dried stem bark of *Bauhinia variegata* (Linn.), were powdered (4Kg) and exhaustively extracted by soxhlet extractor, using 95 % ethanol as solvent. The ethanol was distilled off and the concentrate was evaporated on a water bath to a sticky mass (36g).

The Ethanolic extract of stem bark of *Bauhinia variegata* (Linn.), was subjected to systematic qualitative analysis for the identification of various phyto-constituents.

The Ethanolic extract (30g) was suspended in distilled water (1500 ml) and then extracted with petroleum ether (60 – 80°C, 8 X 500 ml), Benzene (8 X 500 ml), chloroform (8 X 500 ml), ethyl acetate (8 X 500 ml), and methanol (8 X 500 ml), All the fractions were then washed with distilled water (30 ml), dried over anhydrous sodium sulphate and freed of solvent by distillation. The ethanol extract was

thus fractioned into petroleum ether soluble fraction (3.5g), Benzene soluble fraction (2.9g), chloroform soluble fraction (2.5g), ethyl acetate soluble fraction (3.2g), and methanol soluble fraction (6.3g).

The petroleum ether extract (3.1g) was dissolved in CHCl₃ (20 ml) and adsorbed onto silica gel (20 g). After evaporation of the solvent it was loaded onto silica gel column (150 g) prepared in petroleum ether (60–80°C). The column was eluted first with petroleum ether (60-80°C) 100% and continued with petroleum ether (60-80°C): benzene graded mixtures, benzene 100% and benzene: chloroform graded mixtures.

100% chloroform and chloroform: ethyl acetate graded mixtures, ethyl acetate and ethyl acetate: methanol graded mixtures and finally with 100% methanol. The elution was monitored by TLC (Silica gel G; visualization: vanillin-sulphuric acid reagent heated at 110°C and Iodine chamber). Each time 10 ml were collected in a test tube and identical eluates (TLC monitored) were combined and concentrated to 5 ml and kept in a desiccator.

Elution carried out with petroleum ether (100%), fractions 9-16 (KA-01, 45 mg); fractions 29-43 (KA-02, 52 mg); petroleum ether: benzene (95:5), fraction 21-30 (KA-03, 65 mg); petroleum ether: benzene (10:90), fractions 42-49 (KA-04, 57 mg); benzene: chloroform (90:10), fraction 142-145 (KA-05, 65 mg); benzene: chloroform (50:50), fraction 242-252 (KA-06, 70 mg); chloroform (100%), fraction 385-391 (KA-07, 60 mg); were obtained which on further purification by fractional crystallization. Further elution was carried out with various solvents.

The ethanolic extract (5g) was dissolved in CHCl₃ (20 ml) and adsorbed onto silica gel (20 g). After evaporation of the solvent it was loaded onto silica gel column (150 g) prepared

in chloroform. Elution carried out with ethyl acetate (100%), fraction 54-63, compound (KA-08, 35 mg). ethyl acetate: methanol (90:10), fraction 115-121, compound (KA-09, 44 mg).

RESULTS & DISCUSSION

The structures of isolated compounds were elucidated on the basis of R_f , Melting point and spectral data.

Compound KA 01: m.p. 65°C, The compound was isolated as a pale green colour needle shape crystals. R_f value: 0.42 [solvent system; petroleum ether (60-80° C)]. The IR peak at 3447.51 cm^{-1} indicated the presence of OH group, peak at 2922.75 cm^{-1} indicates C-H stretching CH_3 , peak at 1635.2 cm^{-1} indicate C-H stretching CH_2 and peak at 1019.65 cm^{-1} indicates O-H bending. ^1H NMR spectra conclude at δ 3.42 for CH_2 and at δ 2.5 for CH_3 . The Mass spectra showed the molecular ion peak at m/z 552 [M^+] which corresponds to the molecular formula $\text{C}_{37}\text{H}_{76}\text{O}_2$. With the above data and mass spectrum, the compound 01 is identified as **Heptatricontan-12, 13-diol**.

Compound KA 02: m.p. 262-265°C, it showed positive test to Libermann-Burchard's test and salkowski test. R_f value: 0.76 [solvent system-benzene: chloroform (90:10)]. The IR spectrum exhibited strong absorption at 2926 cm^{-1} IR peak at 2929.0 cm^{-1} and 2869.24 cm^{-1} indicating the presence of C-H stretching in CH_3 and CH_2 respectively. IR peak at 1714.96 cm^{-1} for C=O stretching, 1631.8 cm^{-1} indicates $\text{CH}_2\text{-CH}_2$ stretching. Peak at 1462.6 cm^{-1} indicates C-H deformation in gem dimethyl. The ^1H NMR spectra of this compound exhibited the presence of eight methyl group at position in between δ 0.725 to δ 1.179. The ^1H NMR spectra also showed at δ 1.1957 to δ 0.1586 for CH_2 protons. The Mass spectra showed the molecule ion peak at 426 [M^+] corresponding to the molecular formula $\text{C}_{30}\text{H}_{50}\text{O}$. With the above data and mass spectrum, the compound 02 is identified as **Friedelin**.

Compound KA 03: m.p. 74-78°C. The compound was white to cream colour crystal. R_f value: 0.6 [solvent system; benzene: chloroform (80:20)]. IR spectrum showed characteristic peak at 1708.76 cm^{-1} indicated the presence of C=O, 1464.9 cm^{-1} and 1017.8 cm^{-1} indicating the presence of C-H deformation in CH_3 and CH_2 respectively. The peak at 3412.16 cm^{-1} indicated -OH stretching. The ^1H NMR signal at δ 0.85 indicated terminal methyl protons δ 1.2-1.3 indicated CH_2 protons. The Mass spectra showed the molecular ion peak at m/z 340 [M^+] corresponding to the molecular formula $\text{C}_{22}\text{H}_{44}\text{O}_2$. With the above data and mass spectrum, the compound 03 is identified as **n-Docosanoic acid**.

Compound KA 04: Pearl white crystals, m.p.167-170°C. It gave a characteristic colour reaction of sterols. It gave red colour in Salkowski's test and a green colour in Libermann Burchard's test. The peaks at 3351, 2919 and 1665 cm^{-1} in the IR spectra indicated to presence of OH group, C=O stretching and C=C stretching respectively. The peaks at 1462 and 1054 cm^{-1} indicated the presence of deformation of gem dimethyl group and stretching of secondary alcohol functional groups. ^1H NMR spectra showed multiplet at δ 0.714 to 1.046 corresponds to eighteen protons of six methyl groups. Further peaks at δ 1.04 to 2.28 corresponds to eighteen protons of nine - CH_2 - groups and eight protons of -CH. The peaks at δ 5.05 to 5.14 corresponds to olefinic two protons. The broad peak at δ 3.53 and 5.36 corresponds to one proton for -OH group and vinylic proton respectively. ^{13}C NMR spectra showed presence of 29 carbons in the compound. The Mass spectra showed the molecular ion peak at m/z 412 (M^+). The Mass fragmentation was typically to that of **Stigmasterol**.

Compound KA 05: m.p 63-64°C. The compound isolated as colourless crystals. R_f value: 0.79

[solvent system; petroleum ether: benzene (95: 5)]. The IR peak at 3391.1 cm^{-1} indicates the presence of OH group, peak at 2928.0 cm^{-1} indicates C-H stretching in CH_3 , peak at 2860.5 cm^{-1} indicate C-H stretching in CH_2 , and peak at 1707.6 cm^{-1} indicates C=O stretching and peak at 1461.0 cm^{-1} and 1073.9 cm^{-1} indicates C-H deformation in CH_3 and CH_2 respectively. ^1H NMR spectra at $\delta\ 0.7991 - \delta\ 0.9690$ indicates terminal methyl protons, a multiplet was observed in the range $\delta\ 1.0334 - \delta\ 1.3929$ of which corresponds to twenty four protons of CH_2 , NMR signal at $\delta\ 2.0030 - \delta\ 2.05841$ shows a multiplet corresponds to two protons of CH_2 , a triplet was observed in the range of $\delta\ 2.3272 - \delta\ 2.3648$ which corresponds to two protons of CH_2 . The Mass-ESI spectra showed the molecular ion peak at $m/z\ 256$ which corresponding to the molecular formula $\text{C}_{16}\text{H}_{32}\text{O}_2$. With the above data and mass spectrum, the compound V is identified as **n-Hexadecanoic acid**.

Compound KA 06: m.p. $60-62^\circ\text{C}$. It showed positive test to Libermann-Burchard's test and salkowski test. R_f value: 0.71 [solvent system; benzene: chloroform 90:10)]. The peak at 3441.41 cm^{-1} in the IR spectra indicates O-H stretching, peak at 2935.42 cm^{-1} indicates C-H stretching in CH_3 , peak at 2851.86 cm^{-1} indicates C-H stretching in CH_2 , peak at 1736.85 cm^{-1} and 1604.33 cm^{-1} indicates C=O stretching and C=C stretching respectively. Peak at 1464.47 cm^{-1} indicates C-H deformation in CH_3 . In ^1H NMR spectra the signal at $\delta\ 0.6482$ to 1.0110 indicates multiplet corresponds to eighteen protons of six CH_3 groups, signal at $\delta\ 1.076$ to $\delta\ 1.5622$ indicates multiplet which corresponds to twenty two protons of eleven CH_2 groups, signal at $\delta\ 1.8356$ to $\delta\ 2.2871$ indicates multiplet which corresponds to four protons of four CH groups, signal at $\delta\ 3.515$ to 3.538 indicates multiplet which corresponds to one proton of CH group, signal at $\delta\ 5.022$ indicates doublet which corresponds to two protons of CH_2

groups, signal at $\delta\ 5.15$ indicates quadralet which corresponds to two proton of CH_2 groups, signal at $\delta\ 5.34$ indicates hump which indicates one proton of $-\text{OH}$ group. The Mass-ESI spectra showed the molecular ion peak at $m/z\ 597$ corresponding to the molecular formula $\text{C}_{41}\text{H}_{72}\text{O}_2$. With the above data and mass spectrum, the compound 06 was identified as **β -Sitosterol**.

Compound KA 07: m.p. $213-215^\circ\text{C}$. The compound isolated as white colour crystals. Positive test for Liebermann Burchardt test indicated the presence of tetracyclic triterpenoid compound. R_f value: 0.712 [Solvent system, chloroform: methanol (95:5)]. In the IR spectra the broad peak at 3423.44 cm^{-1} indicated the presence of OH, IR peak at 2929 cm^{-1} and 2850 cm^{-1} indicating the presence of C-H stretching in CH_3 and CH_2 respectively, IR peak at 1458.14 cm^{-1} represents CH deformation in gem dimethyl, further peak at 880.00 represents exocyclic CH_2 . In ^1H NMR a multiplet was observed in the range of $\delta\ 0.7605$ to 0.9213 corresponding to twenty one protons of seven methyl groups, a multiplet was observed in the range of $\delta\ 0.9436$ to 1.6811 corresponding to twenty protons of ten CH_2 groups, a singlet was observed at $\delta\ 2.1735$ corresponding to one proton of CH, a multiplet was observed in the range of $\delta\ 3.1645$ to 3.2051 corresponding to one proton of CH group, a triplet was observed in the range of $\delta\ 4.2024$ to 4.2321 corresponding to one proton of CH group, a multiplet was observed in the range of $\delta\ 4.5604$ to 4.5704 corresponding to one proton of CH group, a doublet was observed in the range of $\delta\ 4.6840$ to 4.6898 which corresponds to one proton of CH group, a doublet was observed in the range of $\delta\ 1.6823$ to 1.6908 corresponding to one proton of CH group. The Mass spectra showed the molecular ion peak at $m/z\ 427.4$ corresponding to the molecular formula $\text{C}_{30}\text{H}_{50}\text{O}_2$. With the above data and mass

spectrum, the compound 07 was identified as **Lupeol**.

Compound KA 08: m.p. 95 – 97^oc. The compound was Yellowish solid. R_f value: 0.85 (solvent system; (Methanol 100%). It gave reddish brown colour for Shinoda test. In the IR spectra the broad peak at 3425 cm⁻¹ indicates the presence of OH, peak at 2917 cm⁻¹ shows Ar-H str in aromatic ring, peak at 2848cm⁻¹ shows C-H str in CH₃, peak at 1690 cm⁻¹ shows α, β unsaturated ketone, peak at 1373 cm⁻¹ shows C-H bending in –C(CH₃)₂, peak at 1249 cm⁻¹ shows C-O-C stretching. In ¹H NMR spectra a singlet at δ 6.1, 6.3 and 7.7 corresponds for three Ar-H, doublet at δ 6.8 & 7.5 indicates two proton of Ar-H, humps at δ 8.8, 10.4, 12.4 indicates OH groups corresponds for five protons. The Mass-ESI spectra showed the molecular ion peak at m/z 302 and the other peaks appeared at 261, 216, 170 and 133 corresponding to the molecular formula C₁₅H₁₀O₇. With the above data and mass spectrum, the compound **KA 08** is identified as **Quercetin**.

Compound KA - 09: m.p. 256-258 °C. The compound was brown color crystals. It gave reddish brown colour for Shinoda test. R_f value : 0.71 (solvent system; petroleum ether : benzene 50:50). In the IR spectra the broad peak at 3422.41 cm⁻¹ indicates the presence of OH. The peak at 2958.08 cm⁻¹ indicates C-H stretching in CH₃, peak at 2854.0 cm⁻¹ indicates C-H stretching in CH₂, peak at 1729 cm⁻¹ and 1617.80 indicates C=O stretching and C=C stretching respectively. The IR peak at 1453.50 cm⁻¹

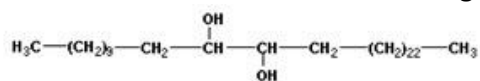
indicates C-H deformation in CH₃. In proton NMR spectra, a triplet at δ 4.0046 indicates two protons of CH, a doublet at δ 4.5151 indicates CH₂ which corresponds to two protons while multiplet ranging from δ 5.8522 to δ 6.3546 and δ 6.3939 to δ 6.5200 indicates aromatic protons corresponds to three hydrogen's and aromatic protons corresponding to one hydrogen respectively. The multiplet ranging from δ 6.6789 to δ 6.8694 and δ 6.9317 to δ 7.5619 represents aromatic protons corresponds to two hydrogens. A signal from δ 8.1878 to δ 8.9660 indicates four -OH group. The Mass-ESI spectra showed the molecular ion peak at m/z 288 corresponding to the molecular formula C₁₅H₁₂O. With the above data and mass spectrum, the compound KA-09 is identified as **Eriodictyol**.

CONCLUSION

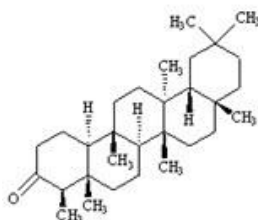
The phytochemical investigation of the petroleum ether and ethanolic extract of the stems of *B. variegata* belonging to the family Caesalpiniaceae was successfully carried out. The chemical constituents isolated from the extracts must be accounted for the biological activities exhibited by the crude petroleum ether and ethanolic extract of the plant. Therefore, it is now turn of the pharmacologists/biologists to explore the plant more systematically by carrying out individual bioactivity of the isolated chemical constituents. Therefore, the present work will boost the scientific communities to do more research work on this important medicinal plant to explore it in the drug development programme going on around the world.

Acknowledgement:

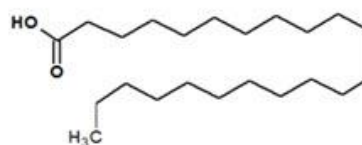
The authors are thankful to Dr. Shivamurthy Murugha Sharanaru, President, & The Executive Directors, SJM Vidyapeetha for providing all necessary facilities through the Principal & HOD of Dept. of Pharmaceutical chemistry, SJM College of Pharmacy, Chitradurga. The authors are also thankful to the students of Dept. of Pharmaceutical Chemistry, SJM College of Pharmacy, Chitradurga.

Structures of the isolated compounds from stem bark of *Bauhinia variegata* Linn.

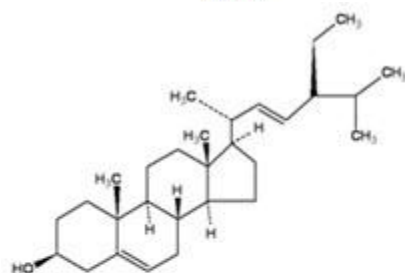
(KA 01)



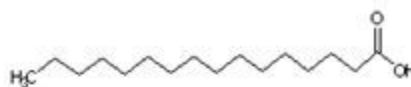
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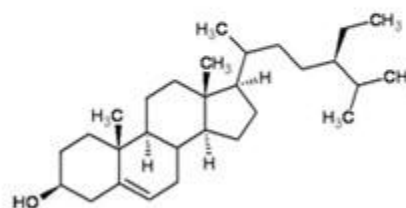
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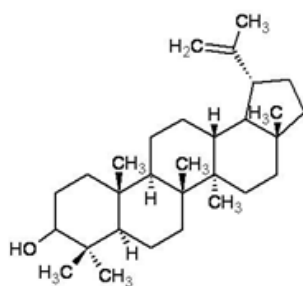
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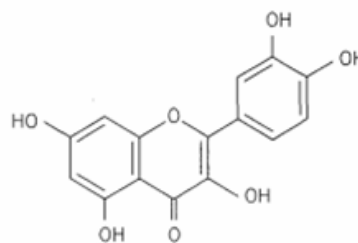
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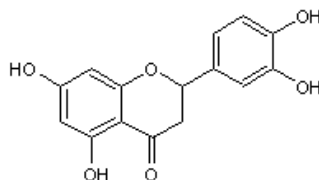
(KA 06)



(KA 07)



(KA 08)



(KA 09)

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