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Phytochemical investigation of the hexane extract of stem bark of *Bauhinia Purpurea* Linn.

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ABSTRACT

The chemical investigation of the stem bark of B. purpurea belonging to the family Leguminosae led to the isolation of nine phytoconstituents namely, Myristic acid, Octadecanoic acid, 9,12-Octadecadienoic acid, isopropyl-24-methyl-pentacosanoate, Stigmasterol, β-Amyrin, β-Sitosterol, Lupeol and ethyl 9,12- Hexadecadienoate. The isolated compounds were characterized using various spectrometric data. These compounds have been reported for the first time from the n-hexane extract of the stem bark of B. purpurea.

Keywords: Myristic acid, Leguminosae, ethyl 9, 12-Hexadecadienoate.

INTRODUCTION

Bauhinia purpurea (Linn.) is a medium sized deciduous tree belonging to the family Leguminosae and subfamily Caesalpinioideae [1]. It is an erect tree with a round, symmetrical, moderate dense crown of 25 feet growing to a height of 20-35 feet tall [2]. The plant is native to India from the foot of the West Himalayas and Khasia mountains growing at an altitude of 4000 feet [3]. The plant is well known for its multiple traditional uses. The bark of the plant is used as an astringent in the treatment of diarrhoea [4-6]. Its decoction is recommended for ulcers as a useful wash solution. The flowers are used as laxative, roots as carminative and leaves as cough suppressant. The bark is used for treating diarrhoea and other gastrointestinal complaints [6]. The bark or root and flowers mixed with rice water are used as maturant for boils and abscesses. The species is thoroughly investigated for the presence of innumerable phytoconstituents and pharmacological properties present in every part of the plant. These findings are already been reported in many cases [7-15]. The aim of the present study is to isolate and characterize the phytoconstituents from the n-hexane extract of stem bark of *B. purpurea* linn.

MATERIALS AND METHODS

PHYTOCHEMICAL INVESTIGATION

General Experimental Procedures: All the melting points were recorded on Model No. BT2-38 melting point apparatus and were uncorrected. IR spectra of the compounds were recorded using the KBr pellet method on a Bruker αT Spectrophotometer. ^{1}HNMR spectra of the compounds were taken on Amx-200 liquid state PMR spectrophotometer using $CDCL_3$ as the solvent. EI-MS data was recorded using EI-MS 2010 PLUS H 200 MHz Shimadzu and LC-MS was recorded using 2010 EV LC-MS Shimadzu instruments at 70eV and 400MHz mass spectrometer. TLC was carried out using Aluchrosep Silica-gel 60/ UV_{254} using precoated plates. Glass Column with a glass stopcock, 30 x 600mm packed with Silica-gel (200-400 mesh) was used for Column Chromatography.

Plant Material: The stem bark of *B. purpurea* was collected from Altinho, Panaji – Goa, India during November, 2011. It was authenticated by Prof. G. I. Hukkeri, Dept. of Botany, Dhempe College of Arts and Science, Miramar – Goa, India.

Preparation of the n-Hexane Extract

The stem bark of *B.* purpurea were taken and dried by means of natural sun drying. The dried bark was powdered (800g) and exhaustively extracted by maceration with n-hexane for three days. After three days, the n-hexane layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off by using rotary flash evaporator. The extract was concentrated to a syrupy consistency and then evaporated to dryness (12g). The preliminary phytochemical studies were performed for testing the different phytoconstituents present in the n-hexane extract. The chemical tests revealed the presence of steroids, triterpenoids, fatty acids and phytol esters.

Isolation of Compounds from n-Hexane Soluble Fraction

The n-hexane soluble fraction (10g) was dissolved in n-hexane (10 ml) and adsorbed onto silica gel (30g). After evaporation of the solvent, the sample was loaded on column packed with 180g of Silica-gel (Molychem, 200-400 mesh) prepared in Petroleum ether (60-80).

The column was subjected to different solvent systems, using Pet. ether (60-80) 100% followed by Pet. ether (60-80): chloroform graded mixtures (95:5, 90:10, 80:20, 70:30, 60:40, 50:50) then with chloroform 100% followed by graded mixtures of Chloroform : ethyl acetate (95:5, 90:10, 80:20, 70:30, 60:40, 50:50) and finally with ethyl acetate 100% and graded mixtures of EtOAc : methanol (99:1, 98: 2, 97:3, 96:4, 95:5, 90:10). The elutions were monitored by TLC (Silica gel-G; visualization by UV 254nm, 366nm and Vanillin-Sulphuric acid spraying reagent heated at 110^0C). Each time 10 ml elutes were collected and identical elutes were combined (TLC monitored) and concentrated to 5ml and kept aside.

Elutions carried out with 100 % Pet. ether (60-80) resulted a single component on TLC (100% Pet. ether (60-80)). After removing the solvent a white semisolid residue resulted. The product was designated as **Compound I** (60mg).
Elutions carried out with graded mixture of Pet. ether (60-80): chloroform (95:05) resulted a single component on TLC (Pet. ether (60-80): chloroform 95:05). After removing the solvent a yellow semisolid residue resulted, which was designated as **Compound II** (36mg).

Elutions carried out with Pet. ether (60-80): chlorofrom (90:10) resulted a single component on TLC (Pet. ether (60-80): chloroform 90:10). After removing the solvent an orange wax resulted, which was designated as **Compound III** (47 mg).

Elutions carried out with Pet. ether (60-80): chlorofrom (80:20) resulted a single component on TLC (Pet. ether (60-80): chloroform 80:20). After removing the solvent a white semisolid resulted, which was designated as **Compound IV** (50mg).

Elutions carried out with Pet. ether (60-80): chlororform (70:30) resulted a single component on TLC (Pet. ether (60-80): chloroform 70:30). The product was designated as **Compound V** (80 mg).

Elutions carried out with Pet. ether (60-80): chloroform (60:40), resulted a single component on TLC (Pet. ether (60-80): chlororform 60:40). The pure compound was designated as **Compound VI** (70mg).

Elutions carried out with Pet. ether (60-80): chlororform (50:50) resulted a single component on TLC (Pet. ether (60-80): chloroform 50:50). The pure compound was further designated as **Compound VII** (53mg).

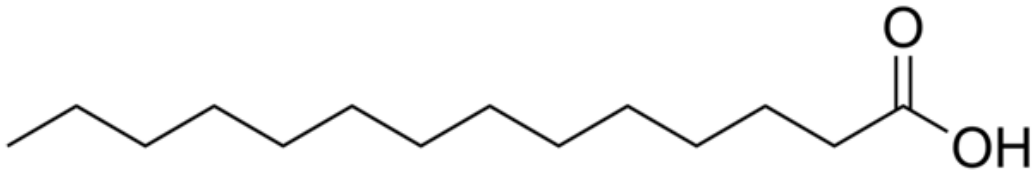
Elutions carried out with 100% chloroform, resulted a single component on TLC (100% chloroform). After removing the solvent, a residue resulted which was further recrystallized with chloroform yielding a white powder. The pure compound was designated as Compound **VIII** (63mg).

Elutions carried out with chloroform: EtOAc (95:05) resulted a single component on TLC (chloroform: EtOAc 95:05). After removing the solvent, a greenish yellow powder resulted. The product was designated as **Compound IX** (40mg).

Elutions carried out with other graded mixtures of solvents resulted in brown resinous mass which was not processed further.

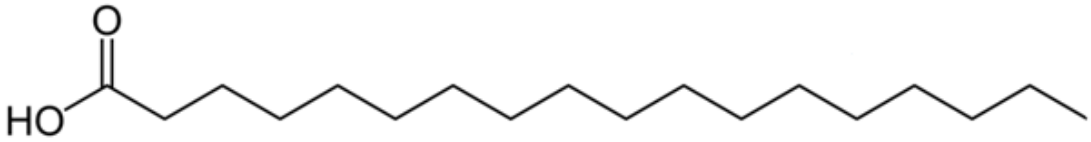
CHARACTERIZATION

Myristic Acid (I): White semisolid; m.p.: 58^{0}C; IR (KBr pellet): ν_{max} 3435.52 cm^{-1} (broad, OH), 2919.10 cm^{-1} (C-H stretching in CH_3), 2855.46 cm^{-1} (C-H stretching in CH_2), 1639.78 cm^{-1} (C = O stretching),1460.20 cm^{-1} (C-H deformation in CH_3); ^{1}HNMR ($CDCl_3$, 200MHz) δ: 0.88 (m, 3H, terminal methyl), 0.910 to 1.255 (m, accounts for 10 x CH_2), 1.328 (s, 2H, (H-3), 1x CH_2), 1.552 (s, 2H (H-2) 1x CH_2). EI-MS: (m/z) 226 $[M+H]^{+2}$ ($C_{14}H_{28}O_2$).



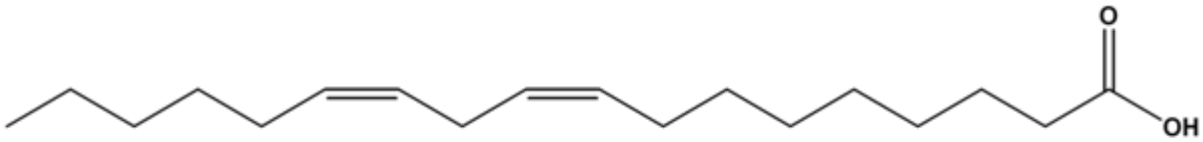
Myristic Acid

Octadecanoic Acid (II): Yellow semisolid; m.p.: 65^{0}C (lit. 69.9^{0}C); IR (KBr pellet) ν_{max} 3421.47 cm^{-1} (broad, OH), 2925.75 cm^{-1} (C-H stretching in CH_3), 2859.03 cm^{-1} (C-H stretching in CH_2), 1718.26 cm^{-1} (C = O), 1455.64 cm^{-1} (C-H deformation in CH_3); ^{1}HNMR ($CDCl_3$) δ: 0.88 (s**,** 3H, terminal methyl group), 2.00 to 2.35 (m, 2H, (H-2), 1x CH_2), 1.60 to 1.67 (m, 2H, (H-3), 1 x CH_2), 1.26 to 1.30 (m, 28 H, 14 x CH_2). EI-MS (m/z): 284 $[M^+ H]^+$ ($C_{18}H_{36}O_2$).



Octadecanoic Acid

9,12-Octadecadienoic Acid (III): Orange wax; IR (KBr pellet) ν_{max} 3411.83 cm^{-1} (broad, OH), 2932.27 cm^{-1} (C-H stretching in CH_3), 1731.69cm^{-1} (C = O stretching), 1453.93 cm^{-1} (C-H deformation in CH_3); ^{1}HNMR ($CDCl_3$) δ: 0.88 (m, 3H, terminal methyl), 0.921 to δ 1.449 (m, 7 x CH_2 for methine groups), 1.681 (s, 2H, (H-3), 1 x CH_2), 2.306 (s, 2H (H-2), 1 x CH_2), 2.344 (s, 4H (H-8, H-14) 2 x CH_2), 2.380 (s, 2H (H-11) 1 x CH_2), 5.360, 5.343 (H, vinylic protons). EI-MS (m/z): 280 $[M]^+$ ($C_{18}H_{32}O_2$).



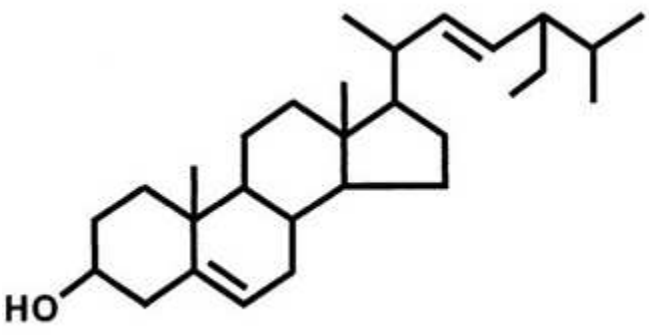
9,12-Octadecadienoic Acid

isopropyl-24-methyl-pentacosanoate (IV): White semisolid; IR (KBr pellet) ν_{max} 2922.73 cm^{-1} (C-H stretching in CH_3), 1729.33 cm^{-1} (C=O str. in COO^-), 1457.22 cm^{-1} (C-H deformation in CH_3), 1377.93cm^{-1} (CH_3 umbrella deformation for iso-propyl group); ^{1}HNMR ($CDCl_3$) δ: 0.846 to 0.957 (m, 12H, 4 x CH_3), 1.256 to 1.306 (m, 40H, 20 x CH_2), 1.449 (s, 1H, H-24), 1.681 (s, 2H, (H-3), 1 x CH_2), 2.344 (s, 2H, (H-2), 1 x CH_2), 4.201 to 4.239 (m, 1H, CH at ester linkage of isopropyl). EI-MS (m/z): 438 $[M]^+$ ($C_{29}H_{58}O_2$).

isopropyl-24-methyl-pentacosanoate

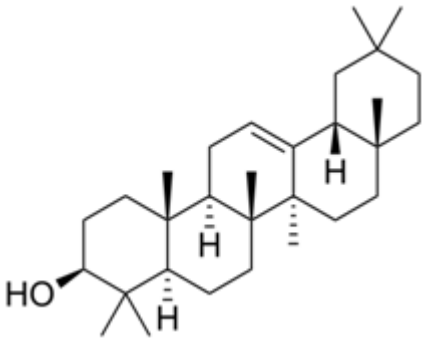
Stigmasterol (V): m.p. 167-170^{0}C. The compound V gave a red colour in Salkowski's test and a green colour in Libermann-Burchard's test specific for steroids. IR (KBr pellet) ν_{max} 3375.38 cm^1 (broad, OH), 2938.10 cm^{-1} (C-H stretching in CH_3), 2837.56 cm^{-1} (C-H stretching in CH_2), 1656.35cm^{-1} (C = C stretching), 1454.48 cm^{-1} (C-H deformation in gem dimethyl groups), 1026.24 cm^{-1} (C-O stretching in secondary alcohol); ^{1}HNMR ($CDCl_3$) δ: 0.975 (s, 3H, H-21), 0.880 to δ 0.911 (m, 9H, H-26, H-27, H-29), 0.847 (s, 3H, H-18), 0.853 (s, 3H, H-19), 1.310

to 1.632 (m, 18H, 9 x CH_2 and 7H, methine protons), 2.77 (s, 1H, H-3), 2 to δ 2.381 (m, 1H, OH), 5.367 (s, 1H, vinylic protons), 5.259 to 5.63 (m, 2H, H-22 and H-23). EI-MS (m/z): 412 $[M]^+$ ($C_{29}H_{48}O$).



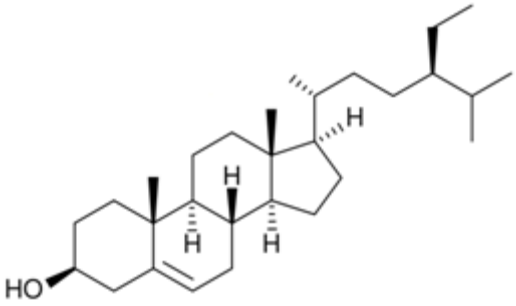
Stigmasterol

β-Amyrin (VI): m.p. 193- 195^{0}C. The compound VI gave a positive response to Libermann-Burchard's test for triterpenoids. IR (KBr pellet) ν_{max} 3390.98 cm^{-1} (broad, OH), 2946.39 cm^{-1} (C-H stretching in CH_3), 2838.01 cm^{-1} (C-H stretching in CH_2), 1651.42 cm^{-1} (C=C stretching), 1455.63 cm^{-1} (C-H deformation in CH_3), 1410.23 cm^{-1} (gem dimethyl stretching), 1111.40cm^{-1} (C-O deformation), 1022.43 cm^{-1} (C-O str. in secondary alcohol); ^{1}HNMR ($CDCl_3$) δ: 0.84 to δ 0.89 (t, 9H, H-24, H-28, H-29), 0.93 to 1.07 (m, 12H, H-23, H-25, H-26, H-30), 1.10 (s, 3H, H-27), 3.66 (s, 1H, H-3), 5.10 to δ 5.36 (s, 1H, H-12, vinylic protons). EI-MS (m/z): 426 $[M]^+$ ($C_{30}H_{52}O$).



β-Amyrin

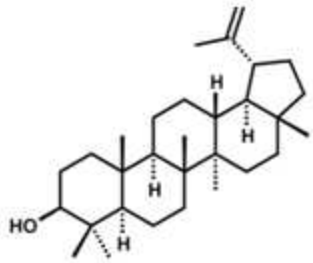
β-Sitosterol (VII): m.p. 138-140^{0}C. The compound VII gave a red colour in Salkowski's test and a green colour in Libermann-Burchard's test. IR (KBr pellet) ν_{max} 3364.08 cm^{-1} (broad, OH), 2941.44 cm^{-1} (C-H stretching in CH_3), 2833.37 cm^{-1} (C-H stretching in CH_2), 1654.97 cm^{-1} (C=C stretching), 1450.03 cm^{-1} (C-H deformation in gem dimethyl), 1027.78cm^{-1} (C-O stretching in secondary alcohol); ^{1}HNMR ($CDCl_3$) δ: 0.85 to1.01 (m, 18H, 6 x CH_3, H-18, H-19, H-21, H-26, H-27, H-29), 1.26 to 1.49 (d, 22H, 11 x CH_2), 1.61 to 2.31 (m, 7H, methine protons), 2.38 (m, 1H, OH), 3.65, 3.67 (m, 1H, C-3), 5.34, 5.37 (s, 1H, H-6, vinylic protons). EI-MS (m/z): 414 $[M]^+$ ($C_{29}H_{50}O$).



β-Sitosterol

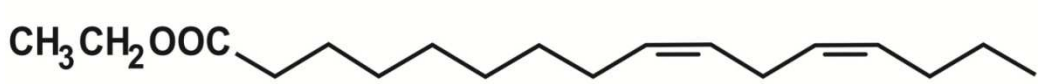
Lupeol (VIII): White powder; m.p. 216-217^{0}C. The compound VIII gave a positive response to Libermann-Burchard's test for triterpenoids. IR (KBr pellet) ν_{max} 3375.38 cm^{-1} (broad, OH), 2938.10 cm^{-1} (C-H stretching in CH_3), 2837.56 cm^{-1} (C-H stretching in CH_2), 1656.35 cm^{-1} (C=C stretching), 1454.48 cm^{-1} (C-H deformation CH_3),

1026.24 cm^{-1} ($CDCl_3$) δ: 0.80 to 1.639 (m, 21H, H-23to H-28, H-30), 2.308 (s, 1H, H-19), 2.804 (s, 1H, H-3), 4.067 to 4.281 (m, 2H, H-29), 5.315 to 5.409 (m, vinylic protons). LC-MS (m/z): 426 $[M]^+$ ($C_{30}H_{50}O$).



Lupeol

ethyl 9,12- Hexadecadienoate (IX): Greenish yellow powder; IR (KBr pellet) ν_{max} 2926.04 cm^{-1} (C-H stretching in CH_3), 2859.04 cm^{-1} (C-H stretching in CH_2), 1713.99 cm^{-1} (C=O stretching of ester), 1457.01 cm^{-1} (C-H deformation in CH_3); ^{1}HNMR ($CDCl_3$) δ: 0.881 (s, terminal methyl group), 2.308, 2.346 (s, 4H [H-8, H-14], 2 x CH_2), 2.382 (s, 2H [H-2], 1 x CH_2), 1.311 (s, 2H [H-3], 1 x CH_2), 2.772 (s, 2H [H-11] 1 x CH_2), 5.259 to 5.421 (m, 2H, vinylic protons), 1.009 to 1.256 (m, accounts for 32H, 16 x CH_2). EI-MS (m/z): 280 $[M]^+$ ($C_{16}H_{27}COO^-$**).**



ethyl 9, 12-hexadecadienoate

RESULTS AND DISCUSSION

Chromatographic gradient elution of the n-hexane extract of stem bark of *B. purpurea* led to the isolation of nine phytoconstituents belonging to the category of steroids, triterpenoids, fatty acids and phytol esters. They were characterized as Myristic acid, Octadecanoic acid, 9,12-Octadecadienoic acid, isopropyl-24-methyl-pentacosanoate, Stigmasterol, β-Amyrin, β-Sitosterol, Lupeol and ethyl 9,12- Hexadecadienoate using spectral analytical techniques like IR, ^{1}HNMR and EI-MS/LC-MS.

CONCLUSION

The phytochemical investigation of the n-Hexane extract of the stem bark of *B.purpurea* linn belonging to the family Leguminosae was successfully completed. The chemical entities isolated and characterised from the stem bark of the plant are Myristic Acid, Octadecanoic Acid, 9, 12- Octadecadienoic Acid, isopropyl-24-methyl-pentacosanoate, Stigmasterol, β-Amyrin, β-Sitosterol, Lupeol and ethyl 9,12- Hexadecadienoate. The compounds were characterized using the evidences obtained from spectral data like IR, ^{1}HNMR and EI-MS/LC-MS. All the above mentioned compounds belong to the class of fatty acids, triterpenoids, steroids and phytol esters.

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