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Short Communication

PHYTOCHEMICAL STUDY OF PETROLEUM ETHER EXTRACT OF STEM BARK OF *BOMBAX MELABARICUM*

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ABSTRACT

Bombax melabaricum DC. is a medium sized tree and distributed mainly in tropical areas of China and hotter part of India. It is used as medicinal herbs. Objective of the study was to isolate active phytochemicals from stem bark of title Plant. Plant Extract of stem bark was chromatographed over silica gel column and eluted with different solvents. Characterization of isolated compounds was done on the basis of spectral studies. Lupeol (I), stigmasterol (II), α-amyrin acetate (III) and trans-triacontanyl-4-hydroxy-3-methoxycinnamate (IV) were isolated. These compounds have very useful medicinal activities. Present work gives a direction for future investigators to get some medicinally important drugs.

Keywords: *Bombax melabaricum,* Bombacaceae, Biological activity, Phytochemicals.

1. INTRODUCTION

Bombax melabaricum DC. (Syn. *Salmalia malabarica DC.*) (BM) is an important medicinal plant of family Bombacaceae [1]. It is a medium sized tree and is commonly known as simul, simbal, silk-cotton tree or Indian kapok [2]. This plant is distributed mainly in tropical areas of China and hotter part of India [3]. Plants of the genus *Bombax* have been used as medicinal herbs from long time. A first regard to its application is reflected in its name, which springs from the Greek words 'taraxis' for inflammation and 'akeomai' for curative. Triterpenes, sterols, acids, sesquiterpene lactones, naphthoquinone derivatives etc. were isolated and characterized from the genus *Bombax*. Prior investigations led to the identification of a sesquiterpene lactone, a potent growth inhibitor in fungi [4] and a flavonol glycoside possessing significant antihypertensive and hypoglycemic activities [5] from BM. Cadinane sesquiterpenoids isolated from BM were found to possess antifungal [6], cytotoxic [7] and anti-HIV activity [8]. It is used as folk medicine because of its demulcent, diuretics, restorative, aphrodisiac and emetic properties [9-11]. The 'semul gum' is used in vata roga (disease of CNS) [12]. It is a component of "Darhamulkulatha" which is employed in diarrhea, dysentery, leucorrhoea and menorrhagia also as for arthritic conditions.

2. MATERIAL AND METHODS

2.1. General experimental procedures

Melting points were determined by electro-thermal melting point apparatus. Qualitative and quantitative TLC was conducted on aluminium sheet Kieselgel 60 F254 (E. Merck). Silica gel (60-120 mesh, 550 gm) used for column (1.5m × 4.0cm) chromatography. The IR spectra were recorded on FT-IR SHIMADZU 8400S spectrometer with KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 MHz and 75 MHz on a Brucker NMR instrument, respectively, using TMS as internal standard. FAB mass spectrums were recorded by JEOL-SX-102/DA-6000 mass spectrometer.

2.2. Plant material

The plant material (stem bark) was collected from the surroundings of Jaipur and the authenticity of the plant was done with the help of Department of Agriculture, Jagan Nath University, Jaipur, Rajasthan India.

2.3. Extraction and Isolation

Shade dried and powdered stem bark of the title plant was extracted with petroleum ether (40-60°C) in a 5.0 liter round bottom flask over water bath for 72 hrs. The extract was filtered and concentrated extract was

chromatographed over silica gel column (60-120 mesh). The column was eluted with increasing order of polarity of solvents in varying compositions. Phytochemicals I to IV were isolated, purified and characterized.

3. RESULTS

3.1. Lupeol (Compound I)

The elution of column with benzene afforded a semi-solid mass after the removal of solvent. This semi-solid mass on crystallization with acetonitrile gave a colourless compound (m.p. 220°C). It gave a yellow color with TNM, showing unsaturation in the compound. IR (KBr, cm^{-1}): 3650-3440, 2950, 1395 and 1360 (gem dimethyl group), 1365. ^{1}H NMR (δ ppm, $CDCl_3$): 0.75 (s, 3H, C^{25}), 0.80 (s, 3H, C^{26}), 0.82 (s, 3H, C^{28}), 0.93 (s, 3H, C^{24}), 0.91 (s, 3H, C^{23}), 1.01 (s, 3H, C^{27}), 1.61 (s, 3H, C^{30}), 4.51 & 4.61 (br, 1H, C^{29}), 2.33 (m, 2H, C^{21}), 3.15 (dd, 2H, *J=11.9, 4.0* Hz, C^3), 1.22-1.69 (m) for remaining 23 protons. ^{13}C NMR (δ ppm, $CDCl_3$): 38.6 (C^1), 27.3 (C^2), 78.7 (C^3), 38.8 (C^4), 55.3 (C^5), 18.1(C^6), 34.3 (C^7), 40.8 (C^8), 54.5 (C^9), 37.0 (C^{10}), 20.9 (C^{11}), 25.2 (C^{12}), 38.01 (C^{13}), 42.5 (C^{14}), 27.4 (C^{15}), 35.9 (C^{16}), 42.8 (C^{17}), 48.2 (C^{18}), 47.9 (C^{19}), 152.1 (C^{20}), 29.8 (C^{21}), 40.0 (C^{22}), 27.9 (C^{23}), 15.3 (C^{24}), 16.1 (C^{25}), 16.0 (C^{26}), 14.5 (C^{27}), 14.5 (C^{28}), 109.1 (C^{29}), 19.1 (C^{30}). MS (m/z): 426 (M^+), 427 $(M+H)^+$, 411, 220, 218, 209 etc. Molecular formula calculated as $C_{30}H_{50}O$

3.2. Stigmasterol (Compound II)

Removal of solvent afforded yellow solid which on crystallisation from chloroform: methanol (1:1) mixture afforded shining crystals, m.p. 166-167°C. It showed single spot on TLC plate. It gave positive Libermann-Burchard and Noller's test for sterols and TNM test for unsaturation. IR (KBr, cm^{-1}): 3400-3200 (OH stretching), 1460 (CH=CH bending). ^{1}H NMR (δ ppm, $CDCl_3$): 5.31 (t, C^6), 5.03 (dd, *J=16, 10* Hz, C^{22}), 5.16 (dd, *J=16, 10* Hz, C^{23}), 3.49 (m, H-3α), 0.85 (t, *J=7* Hz, C^{29}), 1.00 (d, *J=7* Hz, C^{29}), 1.00 (d, *J=7* Hz, C^{21}), 1.15 (s, 3H, C^{27}), 0.94 (C^{19}), 0.68 (C^{18}). MS (m/z): 412 (M^+), 399 $(M\text{-}Me)^+$, 384, 369, 314, 302, 273, 255 etc. Molecular formula calculated as $C_{29}H_{48}O$.

3.3. α-amyrin acetate (Compound III)

By eluting the column with benzene: chloroform (1:1), α-amyrin acetate was isolated. This fraction after removal of solvent was crystallized by acetone as white crystals. (m.p. 223°C). IR (KBr, cm^{-1}): 1739 (O-acetyl group), 1055 (C-O stretching), 1384, 1372. ^{1}H NMR (δ ppm, $CDCl_3$): 0.67 (s, 3H, C^{24}), 0.73 (s, 3H, C^{26}), 0.80 (s, 3H, C^{25}), 5.16 (dd, *J=16, 10* Hz, C^{23}), 0.86 (d, 3H, *J=7.8* Hz, C^{30}), 0.93 (d, 3H, *J=5.7* Hz, C^{29}), 0.98 (s, 3H, C^{23}), 1.04 (s, 3H, C^{28}), 1.12 (s, 3H, C^{27}), 1.25-1.95 (m) (remaining 23 protons), 8.25 (d, *J=6.3* Hz, 1H, C^{12}), 4.47 (t, 3H, *J=6.3* Hz, C^3), 2.04 (s, 3H, $-COCH_3$). ^{13}C NMR (δ ppm, $CDCl_3$): 38.2 (C^1), 24.6 (C^2), 81.4 (C^3), 37.6 (C^4), 56.1 (C^5), 18.6 (C^6), 22.9 (C^7), 40.1 (C^8), 47.9 (C^9), 37.3 (C^{10}), 17.6 (C^{11}), 124.8 (C^{12}), 141.0 (C^{13}), 41.9 (C^{14}), 28.8 (C^{15}), 29.0 (C^{16}), 33.4 (C^{17}), 60.1 (C^{18}), 40.0 (C^{19}), 38.6 (C^{20}), 31.3 (C^{21}), 41.3(C^{22}), 29.0 (C^{23}), 17.3 (C^{24}), 16.1 (C^{25}), 17.7 (C^{26}), 23.4 (C^{27}), 28.2 (C^{28}), 23.7 (C^{29}), 21.6 (C^{30}), 172.3 ($O\underline{C}OCH_3$), 23.7 ($OCO\underline{C}H_3$). MS (m/z): 468 (M^+), 453, 409, 218, 203, 189, 135 etc. Molecular formula calculated as $C_{32}H_{52}O_2$

3.4. trans-triacontanyl-4-hydroxy-3-methoxy-cinnamate (Compound IV)

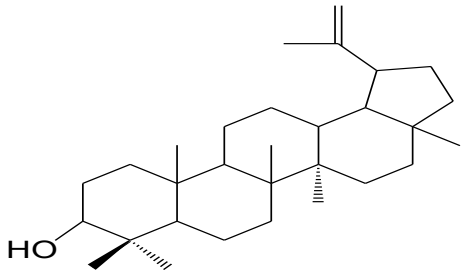
It was isolated as white crystals, m.p. 75°C. Its spectral analysis showed the following results. IR (KBr, cm^{-1}): 3312 (OH group), 1725 (C=O of conjugate ester), 1650 (C=C), 1600, 1517, 1027 (C-O stretch), 980 (C=C trans). ^{1}H NMR (δ ppm, $CDCl_3$): 0.9 (t, 3H, *J=6.4* Hz), 1.25 (brs, 56H), 3.7 (s, $-OCH_3$), 4.3 (t, *J=6.7* Hz, $-CH_2O$), 5.9 (s, 1H, -OH), 6.3 (d, =CH-, *J=15.8* Hz), 7.7 (d, =CH-, *J=15.8* Hz), 6.9 (d, Ar-H, *J=8.6* Hz), 7.1 (dd, Ar-H, *J=8.6, 2.5* Hz), 7.03 (d, Ar-H, *J=2.5* Hz). MS (m/z): 614 (M^+), 177 ($C_{10}H_9O_3$). Molecular formula calculated as $C_{40}H_{70}O_4$.

4. DISCUSSION

4.1. Compound I

Mass spectrum suggested its molecular formula as $C_{30}H_{50}O$, M^+ (426). The compound was found homogenous in TLC. It gave positive Libermann-Burchard and TNM tests which indicated it to be an unsaturated triterpene. The IR spectrum (KBr, cm^{-1}) showed strong absorption at 3635 typical for hydroxyl group. The presence of C=C was confirmed by the characteristic absorption at 1650 cm^{-1}. The sharp absorptions observed at 1395 and 1360cm^{-1} are characteristic for bending vibrations of gem dimethyl group ($-CMe_2$). In the ^{1}H NMR spectrum (δ ppm, $CDCl_3$) of the compound sharp absorptions at 0.75, 0.80, 0.82, 0.93, 0.91 and 1.01 indicated the presence of six methyl groups suggesting the triterpenoid skeleton for this compound. These methyl groups were

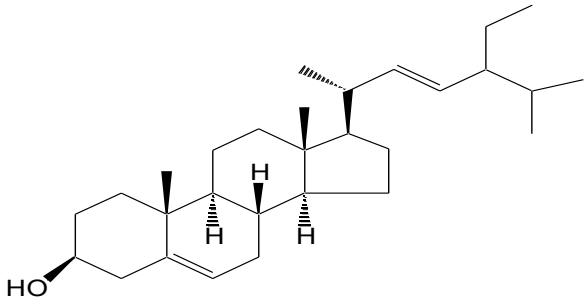
assigned at C^{25}, C^{26}, C^{28}, C^{24}, C^{23} and C^{27} respectively. A broad singlet observed at 1.61 was assigned for three protons of the methyl group attached to the olefinic carbon i.e C^{30} methyl group. The positions of the vinylic protons at C^{29} were observed at 4.51 and 4.61 as a pair of broad singlets. The presence of C^3 proton was detected at 3.15 (*J=11.9, 4.0* Hz) as a doublet and accounted for one proton. This spectral pattern suggested that compound I is a lupane type triterpenoid. A multiplet for two protons at C^{21} was observed at 2.33 of pentacyclic ring. The presence of remaining twenty three protons was confirmed between 1.22-1.69. In the ^{13}C NMR spectrum (δ ppm, $CDCl_3$), the signal observed at 78.8 indicated the presence of a hydroxyl group at C^3 position. The absorptions at 109.1 and 150.1 confirmed the presence of two olefinic carbons at C^{29} and C^{20} respectively. The other ^{13}C NMR values were observed at 14.52 (C^{27}), 15.35 (C^{24}), 14.51 (C^{28}), 15.91(C^{25}), 16.05(C^{26}), 27.94 (C^{23}),18.28 (C^6), 20.91 (C^{11}), 25.2 (C^{12}), 27.38 (C^2), 27.41(C^{15}), 38.68 (C^1), 38.88 (C^4), 55.31(C^5), 34.31(C^7), 40.81 (C^8), 54.5 (C^9), 37.0 (C^{10}), 38.01 (C^{13}), 42.5 (C^{14}), 35.59 (C^{16}), 42.82 (C^{17}), 48.26 (C^{18}), 47.96 (C^{19}), 29.81 (C^{21}), 40.0 (C^{22}), 19.1 (C^{30}). These spectral data was found to be similar to those reported for lupeol [13] and compound I was characterized as lupeol.



Compound I

4.2. Compound II

Isolated as shining needles, m.p 166-167°C. It responded positively for Libermann-Burchard and Noller's test for unsaturation. Its mass spectrum showed a molecular ion peak at m/z 412 $[M]^+$, ($C_{29}H_{48}O$) and other prominent ions were observed at m/z 399, 384, 369, 255 etc. In the IR spectrum (KBr, cm^{-1}) characteristic absorptions displayed at 3400-3200 cm^{-1} (OH stretching) and 1460 cm^{-1} (-CH=CH-bending). The ^1H NMR spectrum (δ ppm, $CDCl_3$) showed a pair of doublets at 5.03 (*J=16* Hz) and 5.16 (*J=16* Hz) which were explainable to proton at C^{22} and C^{23} olefinic side chain. Higher value of *J* (*16* Hz) for these signals designated the trans orientation of consequent protons. A broad triplet at 5.31 was observed for C^6 olefinic proton. Multiplet at 3.49 corresponded to C^3 hydroxy methine proton. The singlets at 0.68 (C^{18}), 0.94 (C^{19}), 1.15 (C^{27}), a doublet centered at 1.00 (*J=7* Hz, C^{21}), and a triplet centered at 0.85 (*J=7* Hz, C^{29}) were observed and assigned. The chemical shifts for C^{18} and C^{19} protons were in close agreement with those reported for Δ^5 sterols. From above spectral data, the compound was characterized as stigmasterol and was confirmed by CO-TLC and m.p. with authentic sample [14].

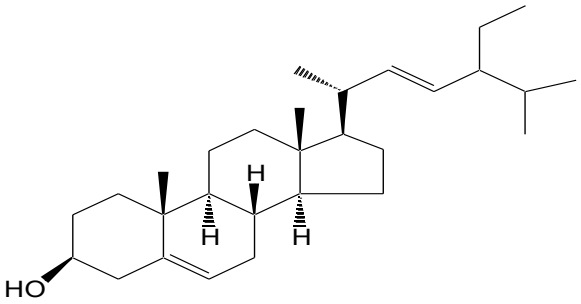


Compound II

4.3. Compound III

On treatment with Libermann-Burchard and Noller's reagent compound III gave positive test showing its triterpenoid nature. Positive test with TNM indicated the unsaturated nature of the compound. With the help of mass spectral observations the molecular formula of the compound III was established as $C_{32}H_{52}O_2$. The molecular ion peak was observed at m/z 468 $[M]^+$ with other prominent peaks at m/z 453, 409, 218, 203, 189, 135 etc. In the IR spectrum (KBr, cm^{-1}) a band at 1739cm^{-1} confirmed the presence of an acetyl group. The presence of C-O stretching was established by appearance of absorption at 1055cm^{-1}. The absorption at 1650=cm^{-1} accounted for C=C stretching. Gem dimethyl group [=C $(CH_3)_2$] appeared as characteristic absorptions at 1384 and 1372 cm^{-1}. In the ^1H NMR spectrum (δ ppm, $CDCl_3$) of compound A, sharp absorptions of three proton each for eight methyl group at 0.80 (δ, 3H, *J=5.7* Hz, C^{25}) 0.86 (d, 3H, *J=7.8* Hz, C^{30}), 0.93 (d, 3H, *J=5.7* Hz, C^{29}), 0.98 (s, 3H, C^{23}), 1.04 (s, 3H, C^{28}), 1.12 (s, 3H, C^{27}), 0.73 (s, 3H, C^{26}), 0.67 (s, 3H, C^{24}) indicated triterpenoid nature for this compound. A triplet observed at 4.47 (*J=6.3* Hz) was accounted to a proton at C^3 position. A characteristic

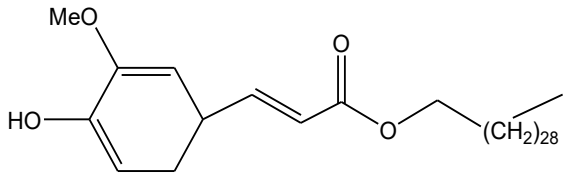
singlet of an acetyl group was observed at 2.04 for three protons at C^3 position. Absorption at 5.25 (d, *J=6.3*) has been assigned to the olefinic proton at C^{12} position. A complicated pattern observed between 1.25-1.95 was ascertained for remaining twenty three protons. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) of compound III showed absorptions at 29.0, 17.3, 16.1, 17.7, 23.4, 28.2, 23.7 and 21.6 for eight methyl groups. The absorption at 81.4 indicated the presence of O-acetyl group attached to the C^3 position. The absorption signal at 23.7 was assigned to methyl carbon of the acetyl group ($COCH_3$) attached to C^3 carbon atom. The absorptions at 124.8 and 141.0 indicated the presence of double bond between C^{12} and C^{13} positions while peak observed at 172.3 is characterized for C=O of acetyl group. Based on exceeding remarks and on comparing these values with the reported data [15] it was identified as α-amyrin acetate.



Compound III

4.4. Compound IV

The mass spectrum of compound IV showed a molecular ion peak at m/z 614[M^+] together with a base peak at m/z 177 for [(OH) (CH_3O) $C_6H_3CH=CHC=O]^+$. On the basis of mass spectral data, ^1H NMR and ^{13}C NMR spectral details its molecular formula was determined as $C_{40}H_{70}O_4$. The IR spectrum (KBr, cm^{-1}) of compound IV confirmed the presence of hydroxyl group by showing a broad absorption at 3312 cm^{-1}. The presence of conjugated ester was established by the absorption at the 1725 cm^{-1}. The proton spectrum (δ ppm, $CDCl_3$) showed the presence of aromatic protons at 7.1 (1H, dd), 6.9 (1H, d) and 7.03 (1H, d). A sharp singlet observed at 3.7 for three protons was ascertained to methoxy group. Presence of sharp singlet at 5.9 was assigned to hydroxyl group. Signals for olefinic protons with trans configuration (6.3 d and 7.7 d) with coupling constant *16* Hz, methylene protons (1.25 brs) and methyl protons (0.9 t) were also appeared. A triplet at 4.3 was given to a methylene group which is attached to oxygen. The above spectral data indicated it to be a trans-triacontanyl-4-hydroxy-3-methoxycinnamate. Its identity was confirmed by comparing these spectral details with those reported on literature.



Compound IV

5. CONCLUSION

Petroleum ether extract of stem bark of *Bombax melabaricum* have a range of different class of natural compounds. These phytochemicals have significant remedial actions so present work provides scope for future investigation to obtain medicinally imperative drugs.

Conflicts of interest

None

6. REFERENCES

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