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Research Article

PHYTOCHEMICAL INVESTIGATION OF THE SILK COCOONS OF *BOMBYX MORI* L.

Kaskoos Raad A., Ali Mohd.*, Naquvi Kamran Javed
Phytochemistry Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India

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*Prof. Mohammed Ali, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi - 110 062, India
E-mail: maliphyto@gmail.com

ABSTRACT
Silk cocoons, produced by *Bombyx mori* L. (Bombicidae) are useful as hypotensive, expectorant, bronchodilator and attenuant drug in traditional medicine. Phytochemical investigation of the ethanolic extract of the cocoons led to the isolation of new phenolic constituents identified as *n*-butyl-3,4-dihydroxybenzoate (**1**), 3′,8′,9′-trigeranilanyl-3,4-dihydroxybenzoate (**2**), 3′,7′-dimethyl-3′-hydroxy-octanyl gallate (**3**), 3,4-dihydroxyphenyl-*n*-pentanyl ether (**4**) and 2,3,4-trihydoxypenyl-*n*-pentanyl ether (**5**) on the basis of spectral data analysis.
Keywords: *Bombyx mori*, silk cocoons, phytoconstituents, phenolic compounds.

INTRODUCTION

Silk cocoons, known as abresham, are produced by a domesticated monophagous insect silk moth, *Bombyx mori* L. (Bombicidae) whose only food is mulberry leaves. The transparent and waxy larva spins its silk cocoon and moves about its head in all directions instinctively feeling for some niche to which the exuding silk fibre can be safely attached[1]. The cocoon shell consists of silk fibroin fibre (70 %) surrounded by a sericin layer made up of sericin (25 %) and nonprotein compounds. The non-sericin component is consisted of carbohydrate, wax, flavonoids and pigments[2-4]. Flavanoids have been found as pigments in the cocoon shells of some silk worm races[5-7]. Larvae of the silk worm sequester flavonoids into their cocoons from the leaves of their host plant, *Morus alba*, family Moraceae[5]. However, flavonol glycosides were not present in the mulberry leaves, but isolated from the cocoons[7]. Therefore, it was inferred that flavonoids absorbed from their diet are modified within the insects by a glucosyl transferase that can transfer a glucose residue to the C-5 hydroxy position of quercetin for using these compounds to increase fitness and to help increase the antioxidative state of tissue[8]. An unsaturated fat with a high concentration of phospholipid was present in the silkworm[9]. In insects the formation of glycosides is the predominant pathway for dietary flavonoids[10,11] and glycosylation of polyphenolics in insects is catalyzed by UDP-glucosyl-transferase (UGT)[12,13] suggesting the possibility that a UGT enzyme that can transfer a glucose moiety to the C-5 position of the flavonoid is functioning in *B. mori*. A correlation between the quantitative changes in L-methionine analogs, the ratio of D-serine and L-serine during the pupal stage, and metamorphosis was observed in the pupae of *B. mori*. [14]. The alkaloid constituents 1-deoxynojirimycine, fagomine and 3-epifagomine were isolated from silkworm dropping[15]. In traditional medicine, ash of the silkworm and cocoon is used as aphrodisiac and rejuvenating tonic[16]. The silk cocoons are useful as hypotensive, expectorant, bronchodilator and attenuant drug and to treat palpitation of heart, cough, asthma and catarrh[17]. This manuscript describes isolation and characterization of phenolic compounds from the silk cocoons produced by *Bombyx mori*.

MATERIALS AND METHODS

General

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded on KBr pellet using a Jasco FT/IR-5000 instrument (FTS 135, Hongkong). The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were scanned on Avance DRX 400, Bruker spectrospin 400 MHz instrument (Rheinstetten, Germany) using $CDCl_3$ as solvent and TMS as internal standard. FAB-MS were measured using JEOL-JMS-DX 303 spectrometer (Peabody, MA, USA). Column (450×4×0.2 cm) chromatography was performed on silica gel (60-120 mesh, Qualigens, Mumbai, India) and thin layer chromatography on silica gel G-coated TLC plates (Merck). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying with ceric sulphate solution.

Plant material

The silk cocoons of *Bombyx mori* were procurred from local market of Delhi, Khari Baoli and authenticated by Dr. Parvez Akhtar, Taxonomist, Central Council for Research in Unani Medicine, Jamia Hamdard (Hamdard University). A voucher specimen No. PRL/JH/08/49 is deposited in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi, India.

Extraction and isolation

The cocoons of *B. mori* were opened to remove the dead pupae. The cocoon shell were dried at 45 ºC for 3 days and coarsely powdered. The powdered cocoon shells (3 kg) were extracted exhaustively with ethanol (95 %) in a Soxhlet apparatus. The ethanolic extract was concentrated under reduced pressure to yield dark yellow, viscous syrupy mass (150 g, 5.0 %). It was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. The slurry was dried in air and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, petroleum ether-chloroform (9:1, 3:1, 1:1, and 1:3 *v/v*), chloroform and chloroform-methanol (99:1, 49:1, 19:5, 9:1, 3:1, 1:1 *v/v*) in order of increasing polarity to isolate the following compounds:

***n*-Butyl protocatechuate (1)**

Elution of the column with petroleum ether furnished pale yellow crystals of **1**; recrystallized from chloroform:acetone (1:1), 150 mg (0.0039 % yield); R_f: 0.73 (chloroform:acetone, 3:2); m.p. 234-235 ºC; UV λ_{max} (MeOH): 224, 271 nm (log ε 5.2, 1.2); IR ν_{max} (KBr): 3450, 2955, 2848, 1725, 1542, 1515, 1210, 980 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.03 (1H, d, *J*=7.8 Hz, H-5), 5.87 (1H, d, *J*=2.7 Hz, H-2), 5.25 (1H, m, H-6), 3.20 (1H, t, *J*=15.0 Hz, H_2-1′), 2.70 (2H, m, H_2-2′), 1.20 (2H, m, H_2-3′), 0.88 (3H, t, *J*=6.5 Hz, Me-4′); ^{13}C NMR (DMSO-d_6): δ 173.81 (C-7), 157.77 (C-3), 157.06 (C-4), 140.11 (C-5), 130.59 (C-1), 128.37 (C-2), 115.44 (C-6), 62.65 (C-1′), 32.81 (C-2′), 29.22 (C-3′), 20.11 (C-4′); +ve FAB MS *m/z* (rel. int): 211 $[M+H]^+$ ($C_{11}H_{15}O_4$) (11.6), 153 (18.3), 137 (32.6).

Geranilantriolyl protocatechuate (2)

Elution of the column with choloroform afforded colourless crystals of **2**, recrystallized from chloroform-acetone, 253 mg (0.0069 % yielded), R_f: 0.66 (chloroform:methanol, 99:1); m.p. 253-255 ºC; UV λ_{max} (MeOH): 205, 277 nm (log ε 0.7, 5.3); IR ν_{max} (KBr): 3450, 3370, 2952, 2845, 1725, 1610, 1550, 1470, 1320, 1110, 980 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.21 (1H, d, *J*=7.8 Hz, H-5), 5.94 (1H, d, *J*=2.7 Hz, H-2), 5.23 (1H, dd, *J*=2.7, 7.8 Hz, H-6), 4.35 (2H, t, *J*=9.5 Hz, H_2-1′), 3.62 (1H, *J*=9.3 Hz, H_2-8a), 3.55 (1H, d, *J*=9.5 Hz, H_2-8b), 3.46 (1H, d, *J*=10.3 Hz, H_2-9a), 3.40 (1H, d, *J*=10.3 Hz, H_2-9b), 2.80 (1H, m, H-9′), 2.51 (2H, brs, H_2-2′), 2.08 (2H, m, H_2-4′), 2.06 (4H, m, H_2-5′, H-6′), 1.26 (3H, brs, Me-10′); ^{13}C NMR (DMSO-d_6): δ 130.56 (C-1), 126.97 (C-2), 157.80 (C-3), 157.05 (C-4), 121.81 (C-5), 115.43 (C-6), 173.84 (C-7), 69.83 (C-1′), 55.88 (C-2′), 71.48 (C-3′), 30.91 (C-4′), 36.07 (C-5′), 29.18 (C-6′), 50.03 (C-7′), 62.65 (C-8′), 64.05 (C-9′), 17.10 (C-10′); +ve FAB MS *m/z* (rel. int): 343 $[M+H]^+$ ($C_{17}H_{27}O_7$) (2.5), 137 (21.6), 153 (30.2), 121 (12.2).

Geranlanolyl gallate (3)

Further elution of the column with choloroform furnished colourless crystals of **3**, recrystallized from chloroform-methanol (1:1), 241 mg (0.0067 % yielded), R_f: 0.68 (chloroform:methanol, 19:1); m.p. 207-208 ºC; UV λ_{max} (MeOH): 223, 256 nm (log ε 4.8, 4.9); IR ν_{max} (KBr): 3422, 3290, 2952, 2844, 1725, 1560, 14758, 1350, 970 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.98 (1H, d, *J*=2.5 Hz, H-2), 7.92 (1H, d, *J*=2.5 Hz, H-6), 4.68 (2H, m, H_2-1′), 2.76 (1H, m, H-7′), 2.50 (2H, brs, H_2-2′), 2.41 (2H, m, H_2-4′), 1.84 (2H, m, H_2-5′), 1.47 (2H, m, H_2-6′), 1.22 (3H, brs, Me-10′), 0.90 (3H, d, *J*=6.5 Hz, Me-8′), 0.84 (3H, d, *J*=6.3 Hz, Me-9′); ^{13}C NMR (DMSO-d_6): δ 140.40 (C-1), 160.08 (C-2), 155.51 (C-3), 151.36 (C-4), 142.57 (C-5), 129.61 (C-6), 171.46 (C-7), 63.05 (C-1′), 50.22 (C-2′), 72.48 (C-3′), 33.67 (C-4′), 31.27 (C-5′), 29.03 (C-6′), 26.58 (C-7′), 22.45 (C-8′), 13.93 (C-9′), 25.19 (C-10′); +ve FAB MS *m/z* (rel. int): 327 $[M+H]^+$ ($C_{17}H_{27}O_6$) (3.2), 153 (45.7), 169 (23.5).

Dihydroxyphenyl-*n*-pentanyl ether (4)

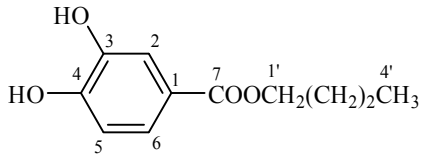
Further elution of the column with choloroform furnished colourless crystals of **4**, recrystallized from acetone, 352 mg (0.0089 % yielded), R_f: 0.77 (chloroform:methanol, 9:1); m.p. 199-201 ºC; UV λ_{max} (MeOH): 221, 263 nm (log ε 4.1, 3.8); IR ν_{max} (KBr): 3415, 3310, 2950, 2845, 1573, 1422, 1205, 1158, 959 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.92 (1H, d, *J*=2.8 Hz, H-2), 7.82 (1H, d, *J*=9.3 Hz, H-5), 7.70 (1H, m, H-6), 3.29 (2H, t, *J*=10.5 Hz, H_2-1′), 2.49 (2H, m, H_2-2′), 1.25 (4H, brs, H_2-3′, H-4′), 0.88 (3H, t, *J*=6.6 Hz, Me-5′); ^{13}C NMR (DMSO-d_6): δ 148.55 (C-1), 144.27 (C-2), 155.25 (C-3), 151.09 (C-4), 140.01 (C-5), 141.77 (C-6), 62.25 (C-1′), 32.53 (C-2′), 28.66 (C-3′), 28.16 (C-4′), 14.27 (C-5′); +ve FAB MS *m/z* (rel. int): 197 $[M+H]^+$ ($C_{11}H_{17}O_3$) (11.9).

***n*-Pentyl isogallyl ether (5)**

Further elution of the column with choloroform yielded colourless crystals of **5**, recrystallized from acetone, 97 mg (0.003 % yielded), R_f: 0.73 (chloroform:methanol, 19:1); m.p. 355-357 ºC; UV λ_{max} (MeOH): 216, 266 nm (log ε 4.8, 2.6); IR ν_{max} (KBr): 3435, 3305, 2955, 2845, 1573, 1422, 1205, 1158, 959 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.93 (1H, d, *J*=8.1 Hz, H-5), 5.82 (1H, d, *J*=8.1 Hz, H-6), 3.41 (2H, t, *J*=8.5 Hz, H_2-1′), 2.50 (2H, t, m, H_2-2′), 1.22 (4H, brs, H_2-3′, H-4′), 0.84 (3H, t, *J*=6.1 Hz, Me-5′); ^{13}C NMR (DMSO-d_6): δ 151.18 (C-1), 157.36 (C-2), 156.28 (C-3), 155.41 (C-4), 148.65 (C-5), 140.16 (C-6), 62.45 (C-1′), 33.41 (C-2′), 29.06 (C-3′), 28.93 (C-4′), 14.16 (C-5′); +ve FAB MS *m/z* (rel. int): 213 $[M+H]^+$ ($C_{11}H_{17}O_4$) (2.3), 141 (11.6).

RESULTS AND DISCUSSION

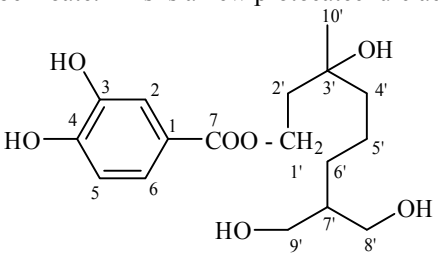
Compound **1**, named *n*-butyl protocatechuate, was obtained as pale yellow crystallize mass from petroleum ether eluents. It gave positive tests for phenols and showed IR absorption bands for hydroxyl groups (3450 cm^{-1}), ester function (1725 cm^{-1}) and aromatic ring (1542, 1515, 980 cm^{-1}). It had a molecular ion peak at *m/z* 211 $[M+H]^+$ ($C_{11}H_{15}O_4$) on the basis of FAB mass and ^{13}C NMR spectra. The ion peaks arising at *m/z* 153 $[M-C_4H_9]$ and 137 $[M-C_4H_9O]^+$ indicated that dihydroxybenzoic acid was esterified with *n*-butanol. The ^1H NMR spectrum of **1** exhibited two one-proton doublets at 7.03 (*J*=7.8 Hz) and 5.87 (2.7 Hz) and a one-proton multiplet at δ 5.25 assigned to aromatic ortho-coupled H-5, meta-coupled H-2 and *ortho*-, *meta*-coupled H-6, respectively. A two-proton triplet at δ 3.20 (*J*=15.0 Hz) was ascribed to oxygenated methylene H_2-1′ protons. A three-proton triplet at δ 0.88 (*J*=6.5 Hz) and two two-proton multiplets at δ 2.70 and 1.20 were attributed primary methyl H_3-4′ and methylene H_2-2′ and H_2-3′, respectively. The ^{13}C NMR spectrum of **1** displayed signals for ester carbon at δ 173.81 (C-7), aromatic carbons between δ 157.77-115.44, oxygenated methylene at δ 62.65 (C-1′), methylene carbons at δ 32.81 (C-2′) and 29.22 (C-3′) and methyl carbon at δ 20.11 (C-4′). Acid hydrolysis of **1** yielded protocatechuic acid, m.p. 198-200 ºC, co-TLC comparable. These data led to characterized the structure of **1** as *n*-butyl-3,4-dihydroxybenzoate.



1

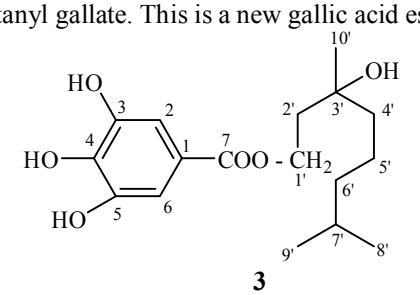
Compound **2**, named geranilantriolyl protocatechuate, was obtained as colourless crystals from chloroform eluents. It gave positive tests for phenols and showed IR absorption bands for hydroxyl groups (3450, 3370 cm^{-1}), ester function (1725 cm^{-1}) and aromatic ring (1550, 980 cm^{-1}). It had a molecular ion peak at *m/z* 343 $[M+H]^+$ ($C_{17}H_{27}H_7$) determined on the basis of FAB mass and ^{13}C NMR spectra. The ion peaks arising at *m/z* 153 [$C_{1'}$-O fission, $C_6H_3(OH)_2COO]^+$ and 137 [C_7-O fission, $C_6H_3(OH)_2CO]^+$ indicated that dihyroxybenzoic acid was esterified with tetrahydroxygeranilanol. The ^1H NMR spectrum of **2** showed two one-proton doublets at δ 7.21 (*J*=7.8 Hz) and 5.94 (*J*=2.7 Hz) and a one-proton double doublet at δ 5.23 (*J*=2.7, 7.8 Hz)

assigned to aromatic H-5, H-2 and H-6 protons, respectively. Four one-proton doublets at δ 3.62 (J=9.3 Hz) and 3.55 (J=9.5 Hz) and at δ 3.46 (J=10.3 Hz) and 3.40 (J=10.3 Hz) were ascribed to hydroxyl methylene H_2-8′ and H_2-9′. A two-proton triplet at δ 4.35 (J=9.5 Hz) was accounted to oxygenated methylene H_2-1′. The signals between δ 2.80-2.06 were due to methine H-7′ and the methylene H_2-2′, H_2-4′, H-5′ and H-6′ protons. The ^{13}C NMR spectrum of **2** displayed signals for ester carbon at 173.84 (C-7), benzene carbons between δ 157.80-115.43, oxygenated methylene carbons at 69.83 (C-1′), 62.65 (C-8′) and 64.05 (C-9′), hydroxyl quaternary carbon at δ 71.48 (C-3′) and methyl carbon at 17.10 (C-10′). Acid hydrolysis of **2** yielded protocatechuic acid, m.p. 199-200 ºC, as one of the component. On the basis of these evidences the structure of **2** has been formulated as 3′,8′,9′-trihydroxy geranilanyl-3,4-dihydroxybenzoate. This is a new protocatechuic acid ester.



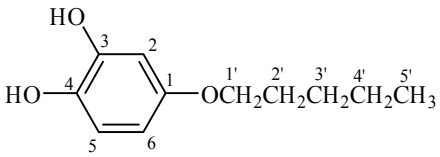
2

Compound **3**, named geranlanolyl gallate was obtained as colourless crystalline mass from chloroform eluents. It gave positive tests for phenols and showed IR absorption bands for hydroxyl groups (3422, 3290 cm^{-1}), ester function (1725 cm^{-1}) and aromatic ring (1560, 970 cm^{-1}). It had a molecular ion peak at m/z 327 $[M+H]^+$ ($C_{17}H_{27}O_6$) determined on the basis of FAB mass and ^{13}C NMR spectra. The ion peaks arising at m/z 169 $[C_6H_3(OH)_3COO]^+$ and 153 $[C_6H_3(OH)_3CO]^+$ indicated that gallic acid was esterified with geranlandiol-type monoterpenes. The ^{1}H NMR spectrum of **3** showed two one-proton doublets at δ 7.98 (J=2.5 Hz) and 7.92 (J=2.5 Hz) assigned to meta-coupled H-2 and H-6, respectively, a one proton multiplet at δ 4.68 ascribed to oxygenated methylene H_2-1′ proton, a three-proton broad signal at δ 1.22 attributed to tertiary C-10′ methyl protons, two three-proton doublets at δ 0.90 (J=6.5 Hz) and 0.84 (J=6.3 Hz) accounted to secondary C-8′ and C-9′ methyl protons, respectively, and other methine and methylene protons between δ 2.76-1.47. The ^{13}C NMR spectrum of **3** exhibited signals for ester carbon at δ 171.46 (C-7), aromatic carbons between 160.08-129.61, oxygenated methylene carbon at 63.05 (C-1′), tertiary alcoholic carbon at δ 72.48 (C-3′) and methyl carbons at δ 22.45 (C-8′), 13.93 (C-9′) and 25.19 (C-10′). Acid hydrolysis of **3** yielded gallic acid, m.p. 235-237 ºC (co-TLC comparable). On the basis of above discussion, the structure of **3** has been characterized as 3′,7′-dimethyl-3′-hydroxyoctanyl gallate. This is a new gallic acid ester.



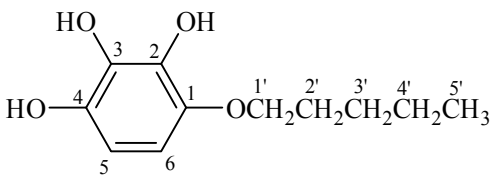
3

Compound **4**, named dihydroxyphenyl n-pentanyl ether, was obtained as colourless crystals from chloroform eluents. It responded to phenolic tests positively and showed IR absorption bands for hydroxyl groups (3415, 3310 cm^{-1}) and aromatic ring (1573, 959 cm^{-1}). It had a molecular ion peak at m/z 197 $[M+H]^+$ ($C_{11}H_{17}O_3$) established on the basis of FAB mass and ^{13}C NMR spectra. Its ^{1}H NMR spectrum exhibited two one-proton doublets at δ 7.92 (J=2.8 Hz) and 7.82 (J=9.3 Hz) and a one-proton multiplet at δ 7.70 assigned to aromatic meta-coupled H-2, *ortho*-coupled H-5 and ortho-, meta-coupled H-6, respectively, a two-proton triplet at δ 3.29 (J=10.5 Hz) ascribed to oxygenated methylene H_2-1′, other methylene protons at δ 2.49 (2H) and 1.25 (4H) and a three-proton triplet at δ 0.88 (J=6.6 Hz) accounted to C-5′ primary methyl protons. The ^{13}C NMR spectrum of **4** showed signals for aromatic carbons between δ 155.25-140.01, oxygenated methylene carbon at δ 62.25 (C-1′); other methylene carbons at δ 32.53 (C-2′), 28.66 (C-3′) and 28.66 (C-4′) and methyl carbon at δ 14.27 (C-5′). On the basis of the above discussion, the structure of **4** has been characterized as 3,4-dihydroxyphenyl-n-pentanyl ether. This is new aromatic ether.



4

Compound **5**, designated as n-pentyl isogallyl ether, was obtained as colourless crystals from chloroform eluents. It gave positive tests for phenol and showed IR absorption bands for hydroxyl groups (3435, 3305 cm^{-1}) and aromatic ring (1573, 959 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peak was determined at m/z 213 $[M+H]^+$corresponding to a phenolic ether ($C_{11}H_{17}O_4$). The ion peak arising at m/z 141 [O-C$_{1'}$ fission, $C_6H_2(OH)_3O]^+$ indicated that tetrahydroxyphenyl ring was involved in ether formation. The ^{1}H NMR spectrum of **5** exhibited signals as doublets at δ 6.93 (J=8.1 Hz) and 5.82 (J=8.1 Hz) assigned to *ortho*-coupled aromatic H-5 and H-6, respectively, a two-proton triplet at δ 3.41 (J=8.5 Hz) ascribed to oxygenated methylene H_2-1′ protons, two signals at δ 2.50 (2H, m) and 1.22 (4H, brs) due to other methylene protons and a three-proton triplet at δ 0.84 (J=6.1 Hz) accounted to primary methyl C-5′ protons. The ^{13}C NMR spectrum of **5** showed the presence of aromatic carbon signals between δ 157.36-140.16, oxygenated methylene carbon at δ 62.45 (C-1′), other methylene carbons at δ 33.41 (C-2′), 29.06 (C-3′) and 28.93 (C-4′) and methyl carbon at δ 14.16 (C-5′). The data led to establish the structure of **5** as 2,3,4-trihydroxyphenyl-n-pentanyl ether. This is a new aromatic ether.



5

CONCLUSION

Five new phytoconstituents have been isolated from the silk cocoons of *Bombyx mori* which may be responsible for the medicinal properties of drug.

ACKNOWLEDGEMENT

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