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Research Article

Isolation of Orientin and Vitexin from Stem Bark of *Parkinsonia aculeata* (*Caesalpiniaceae*) and their Successive Blending on Sheep Wool Fiber

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ABSTRACT
The benzene fraction from defatted ethanol extract of the stem bark of *Parkinsonia aculeata* (Caesalpiniaceae) afforded α-amyrinacetate (I), β-amyrinacetate (II), α-amyrin (III), 6-hydroxytritriacontan-3-one (IV), 6-hydroxypentacosylpentanoate (V), ethylnonadecanoate (VI), orientin (VII) and vitexin (VIII). The structures of isolated compounds were ascertained using various spectral (IR, ^{1}HNMR, ^{13}C NMR, MS) techniques. Compound VII and VIII were examined as natural dye constituents over sheep wool fibers furnished yellow and brown colours, respectively on wool fibers with good fastness properties.

Keywords *Parkinsonia aculeata*, Caesalpiniaceae, α-amyrinacetate (I), β-amyrinacetate (II), α-amyrin (III), 6-hydroxytritriacontan-3-one (IV), 6-hydroxypentacosylpentanoate (V), ethylnonadecanoate (VI), orientin (VII), vitexin (VIII), sheep wool fibres.

INTRODUCTION

P*arkinsonia aculeata* (C*aesalpiniaceae*) is a large spinous shrub or a small tree up to 4 m in height, with edible seeds[1] and distributed throughout the Indian plains and native of tropical America[2,3]. The alcoholic extract of its bark exhibited central nervous system depressant activity in mice where as aqueous extract showed cholinomimetic activity[4]. Literature survey of the genus *Parkinsonia* showed the presence of flavones[5], flavone C-glycoside[6-9], furfural contents[10], carbohydrate[11], free amino[12] and fatty acids[13]. The present study deals with the isolation and characterization of α-amyrinacetate (I), β-amyrinacetate (II), α-amyrin (III), 6-hydroxytritriacontan-3-one (IV), 6-hydroxypentacosylpentanoate (V), ethylnonadecanoate (VI), orientin (VII) and vitexin (VIII) from the benzene fraction of the defatted ethanol extract of the stem bark of *Parkinsonia aculeata* and the compounds *viz*. orientin (VII) and vitexin (VIII) evaluated for their dying properties on sheep wool fibres.

MATERIALS AND METHODS

IR spectra were recorded on FTIR Nicolet Magna 550 and Schimadzu 8400 S spectrometers. ^{1}H and ^{13}C NMR spectra were measured on JEOL AL 300 MHz FTNMR instrument. Mass spectra (FAB MS) were generated on a JEOL SX-102 spectrometer. UV-VS spectra were recorded in methanol (95%) on Perkin Elmer model 2R automatic recording. Optical rotations were measured on JAS CODIP-370 digital polarimeter. Qualitative and quantitative TLC's were conducted on aluminium sheets Kieselgel 60F_{254} (E. Merck). Melting points were determined in soft glass capillaries in an electro thermal melting point apparatus and are uncorrected. The colourimetric values were evaluated using a JAYPAK4802 Colour matching system (Jay Instruments Ltd, Mumbai, India) at D65 illuminate/ 10° observer. The washing and light fastnesses of the dyed samples was evaluated as per standard procedure ISO 1005-A02 and BS 1006 (B02) respectively.

Extraction and Isolation of constituents from the stem bark of *Parkinsonia aculeate:* Shade dried stem bark (2 kg) was coarsely powdered and extracted with ethanol on a steam bath for 72 hrs. The extract was concentrated under reduced pressure. The syrupy mass so obtained was treated with acetonitrile for the removal of fats. The fat free extract was extracted with benzene, the solvent was removed under reduced pressure. The rest mass (48 g) was subjected to silica gel column chromatography and eluted with the solvents of increasing polarity**.** Fraction I was eluted with petroleum ether: benzene (1:3) afforded a light yellow solid mass (4 g) obtained after removal of solvent and the compound I (3.4 g) was crystallized with ethyl acetate-methanol as white needles. The fraction II eluted with petroleum ether: benzene (1:1) followed by crystallization from chloroform-methanol mixture gave white needles as compound II (4.2 g). Fraction III eluted with petroleum ether: benzene (1:1) obtained as a colourless solid (3.6 g) after removal of solvent which appeared as a trail on a silica gel microchromatoplate using petroleum ether :

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Table 1: Colourimetric and fastness properties of orientin dyed fabrics

S.No	Sample details	L*	a*	b*	Washing Fastness	Light Fastness
1	Control fabric	85.04	0.34	7.92	-	-
2	Dyed with orientin alone	58.35	18.87	6.82	3-4	6-7
3	Dyed with orientin + aluminium sulphate	58.49	18.60	6.01	3	7
4	Dyed with orientin + stannous chloride	56.95	18.54	5.68	3	6-7
5	Dyed with orientin + ferrous sulphate	53.44	17.43	6.32	3	7

benzene (1:1) as developer. It was purified as compound III (3.2 g) by repeated crystallization from ethyl acetate-methanol in the form of white needles. The fraction IV eluted with petroleum ether: benzene (1: 9) followed by crystallization from ethyl acetate gave a white powder as compound IV (3.8 g). Fraction V eluted with solvent benzene: chloroform (3:1) yielded a white residue (5 g) on evaporating the solvent and crystallized with chloroform-methanol afforded a white amorphous powder as compound V (4.2 g). Fraction VI eluted with benzene: chloroform (1:1) gave a white solid (4.8 g) after evaporation of the solvent and compound VI (4.2 g) was crystallized with chloroform-methanol (2:3) as white plates. Compound VII (5.8 g) was obtained as yellow solid when the column was eluted with acetone: methanol (4:1) and crystallized with chloroform and methanol mixture. On elution of the column with acetone: methanol (2:3), compound VIII (5.4 g) was isolated as brown flakes after crystallization with chloroform methanol mixture.

α-Amyrinacetate: colourless needles, m.p. 222- 23 ^{O}C; IR (ν_{max}) (KBr) (cm^{-1}) : 2925, 2850, 1730, 1650, 1385, 1370, 1250, 1050, 900 etc; ^{1}H NMR (300 MHz, $CDCl_3$) (δ, ppm) : 0.80 s (3H), 0.83 s (3H), 0.87 s (3H), 0.91 s (3H), 0.97 s (3H), 0.98 s (3H), 1.01 s (3H), 1.07 s (3H), 1.13 to 1.90 (remaining 23 protons), 2.06 s (3H), 4.50 t (1H), 5.12 s (1H); ^{13}C NMR (75.45 MHz, $CDCl_3$) (δ, ppm) : 39.0 (C-1), 28.1 (C-2), 80.2 (C-3), 37.0 (C-4), 54.6 (C-5), 17.6 (C-6), 33.1 (C-7), 40.0 (C-8), 47.0 (C-9), (C-10), 23.1 (C-11), 121.7 (C-12), 138.9 (C-13), 41.4 (C-14), 26.2 (C-15), 27.5 (C- 16), 34.1 (C-17), 58.4 (C-18), 39.5 (C-19), 39.8 (C-20), 31.9 (C-21), 40.9 (C-22), 27.8 (C-23), 16.2 (C-24), 15.1 (C-25), 16.1 (C-26), 22.9 (C-27), 29.0 (C-28), 16.9 (C-29), 20.8 (C-30), 170.2 (C=O), 23.1 (CH_3); MS : (*m/z*) 468 [M$^+$] ($C_{32}H_{52}O_2$), 453, 409, 393, 271, 231, 218, 203, 189, 175, 161, 147, 135, 119, 105, 8 0.

β-Amyrinacetate : colourless flakes, m.p. 236-37 ^{O}C; IR (ν_{max}) (cm^{-1}) (KBr) : 2950, 2850, 1730, 1660, 1462, 1380, 1360, 1240; Mass : (*m/z*) 468 [M$^+$] ($C_{32}H_{52}O_2$), 453, 423, 409, 397, 339, 323, 311, 297, 283, 271, 218, 135, 105, etc.

α-Amyrin : colourless needles, m.p. 183- 84 ^{O}C; IR (ν_{max}) (cm^{-1}) (KBr) : 3350 (OH), 2990 - 2850, 1640, 1480, 1380, 1360, 1200, 1130, 1050, 1030, 990, 960, 930, 890 and 820; ^{1}H NMR (300 MHz, $CDCl_3$) (δ, ppm) : 0.76 s (3H), 0.79 s (3H), 0.83 s (3H), 0.87 s (3H), 0.89 s (3H), 0.99 s (3H), 1.02 s (3H), 1.07 s (3H), 1.25 to 2.0 (24 protons) 3.5 t (H); Mass : *m/z* 426[M$^+$] ($C_{30}H_{50}O$), 410, 399, 341, 327, 313, 281, 267, 231, 189, 147, 135, 121, 105, etc.

6-Hydroxytritriacontan-3-one : white granules, m.p. 71-72 ^{O}C; IR (ν_{max}) (cm^{-1}) (KBr) : 3350 (OH), 2900-2830 (CH), 1720 (C=O), 1480, 1370, 1090, 720 and 710; Mass : *m/z* 494 [M$^+$] ($C_{33}H_{66}O_2$), 465, 451, 437, 423, 409, 394, 379, 366, 352, 338, 324, 310, 296, 282, 268, 254, 226, 197, 169, 141, 127, 115, 99, 85, 71, 57 etc.

6-Hydroxypentacosylpentanoate : white granules, m.p. 83-84 ^{O}C; IR (ν_{max}) (cm^{-1}) (KBr) : 3390 (OH), 2920-2840 (CH), 1730 (-COO-), 1475, 1465, 1185, 1135, 730, 725 and 710; ^{1}H NMR (300 MHz, $CDCl_3$) (δ, ppm) : 4.11 t (2H, $CO\text{-}O\text{-}CH_2$) 3.55 m (2H, -CH-OH), 2.24 t (2H, CH_2-CO), 1.18 t (48H, 27 × -CH_2) and 0.81 t (6H, 2 × Me); Mass : *m/z* 468 [M$^+$] ($C_{30}H_{60}O_3$), 439, 425, 411, 383, 277, 267, 253, 201, 171, 85, 57 etc.

Ethylnonadecanoate : white plates, m.p. 72-73 ^{O}C; IR (ν_{max}) (cm^{-1}) (KBr) : 2960-2890, 1740 (-COO-), 1470, 1180, 730 and 720; Mass : *m/z* 326 [M$^+$] ($C_{21}H_{42}O_2$), 314, 207, 280, 252, 269, 254, 241, 195, 167, 139, 125, 111, 97, 83, 71, 57 etc.

Orientin : yellow solid, m.p. 346 ^{O}C; R_f: 0.63 [Ethyl acetate: formic acid: glacial acetic acid: water (100: 11: 11: 27)]; UV (λ_{max}, nm) (MeOH) : 255, 267,293, 346.

Vitexin : brown flakes, m.p. 367 ^{O}C; R_f : 0.63 [Ethyl acetate: formic acid: glacial acetic acid: water (100 : 11: 11: 27)]; UV (λ_{max}, nm) (MeOH) : 270,302, 336.

Fabric and dyeing: Sheep fabric with 91 g/ m^2 areal density was used for this study. The fabric was mild scoured with a 0.5 g/L nonionic detergent. The scoured fabric was then dyed with 5% colourant (own) at 90 ±2 ^{O}C with 1: 40 material to liquor ratio at pH 5-6 in presence of 0.5 g/L acetic acid solution for one hour in a water bath. After dyeing, the samples were taken out and 3% (owm) solution of mordants ($Al_2(SO_4)_3$, $SnCl_2$, and $FeSO_4$) were added and continued for another one hour. The fabrics were then rinsed with water and dried at ambient condition.

RESULTS AND DISCUSSION

Chemical examination of the stem bark of *Parkinsonia aculeate:* The benzene fraction of the defatted ethanol extract prepared from stem bark of *Parkinsonia aculeata* was chromatographed over silica gel column and compounds I-VIII were isolated and characterized as follows.

Compound I colourless needles, m.p. 222-23 ^{O}C analyzed for molecular formula $C_{32}H_{52}O_2$, [M$^+$, 468] from its FAB mass spectrum. It furnished yellow colour with TNM indicating unsaturation in the molecule and colour reactions characteristic of triterpenoid compounds, *viz*. Liebermann-Burchard reaction[14] and Noller's reaction[15]. In the infrared spectrum, peaks appeared at 1730 (C=O stretch), 1650 (C=C stretch) and 1250 cm^{-1} (O=C-O) for acetate moiety. In ^{13}C NMR spectrum the absorption at δ_C 170.2 (C=O) and δ_C 23.1 ($COCH_3$). The absorption at δ_C 80.2 also indicated that acetyl group is attached to the C-3.

Table 2: Colourimetric and fastness properties of vitexin dyed fabrics

S.No	Sample details	L*	a*	b*	Washing Fastness	Light Fastness
1	Control fabric	85.06	0.35	7.96	-	-
2	Dyed with vitexin alone	58.37	18.88	6.86	4-5	4-5
3	Dyed with vitexin + aluminium sulphate	58.51	18.61	6.06	4	5
4	Dyed with vitexin + stannous chloride	56.97	18.55	5.70	4	4-5
5	Dyed with vitexin + ferrous sulphate	53.46	17.44	6.34	4	5

The protons present at C-3 appeared as triplet at δ_H 4.50. Absorption at δ_H 5.12 has been assigned to one olefinic proton at C-12. It was also supported by the absorption at δ_C 121.7 and δ_C 138.9 in ^{13}CNMR spectrum, assigned to C-12 and C-13 carbon atoms respectively. In ^{1}H NMR spectrum the eight singlets for the methyl groups were observed at δ_H 0.80 (C-24), 0.83 (C-25), 0.87 (C-26), 0.91 (C-29), 0.97 (C-30), 0.98 (C-27), 1.01 (C-23), and 1.07 (C-28). A complicated pattern was observed between δ_H 1.13 and δ_H 1.90 accounting for twenty three alicyclic protons. In the mass spectrum, the molecular ion peak was observed at *m/z* 468. Intense peaks appeared at 453 [M^+-CH_3], 409 [M^+-CH_3COOH], 393, 218, 203 and 189 indicating it to be an ursane type triterpenoid. On the basis of above observations along with the reported data it was identified as α-amyrin acetate[16]. Compound II, colourless needles, m.p. 236-37 ^{O}C was analyzed for molecular formula $C_{32}H_{52}O_2$, *m/z* 468 [M^+] from its FAB mass spectrum. It exhibited yellow colour with TNM indicating unsaturation in the molecule and characteristic colour reactions (deep red violet) for triterpenoid compounds *viz*. Liebermann-Burchard[14] and Noller's reactions[15]. The important peaks observed in the infrared spectrum were at 1730 (C=O stretch), 1660 (C=C stretch) and 1240 cm^{-1} (O=C-O of acetate). Besides the molecular ion peak other important peaks appeared at 453 [M^+-Me], 409 [M^+-AcOH], 397, 218, 203 and 189.Hence, compound II was characterized as β-amyrin acetate[17]. Compound III, colourless needles, m.p. 183-84 ^{O}C and molecular formula $C_{30}H_{50}O$, [M^+, 426] was arrived from its FAB mass spectrum. It produced yellow colour with TNM indicating unsaturation in the molecule and showed colour reactions of triterpenoid compounds. In the infrared spectrum, absorption bands at 3350 and 1130 cm^{-1} indicated the presence of a secondary hydroxyl group and at 1640 cm^{-1} indicated unsaturation in the molecule. ^{1}H NMR spectrum showed absorptions singlets at δ_H 0.76, 0.79, 0.83, 0.87, 0.89, 0.99, 1.02 and 1.07 indicating the presence of eight methyl groups which suggest that the compound should be a pentacyclic triterpene. The proton attached at C-3 position, observed as a triplet at δ_H 3.5 confirming that hydroxyl group is attached to the same carbon atom. Remaining twenty four protons showed absorption between δ_H 1.25 and δ_H 2.0. In the mass spectrum, the molecular ion peak was observed at *m/z* 426 [M^+]. The base peak appeared at *m/z* 105 and peak at *m/z* 218 $[C_{16}H_{26}]^+$ corresponded to the ion resulting from the retro-Diels-Alder cleavage of the molecular ion. The other intense peaks were observed at *m/z* 410 [M^+-Me], 341, 327, 313, 281, 267, 231, 189, 135, 105 etc. Above discussions concluded that the compound III was an α-amyrin[18-20]. Compound IV white granules, m.p. 71-72 ^{O}C and molecular formula $C_{33}H_{66}O_2$, [M^+, 494] followed from its FAB mass spectrum. In the IR spectrum, a broad peak at 3350 cm^{-1} and intense peak at 1720 cm^{-1} indicated the presence of hydroxyl and ketonic groups, respectively. Its molecular ion peak appeared at *m/z* 494 [M^+] and appearance of abundant ions were at *m/z* 465 and 437 due to α-cleavage led to the assignment of carbonyl group at C-3. Loss of a fragment of 30 mass units from fragment *m/z* 409 to give a fragment of *m/z* 379 indicated the position of hydroxy group at C-6 together with many peaks with uniform loss of 14 mass units. The straight chain nature of the compound was confirmed by the absence of an [M^+-15] ion. Thus, the compound IV characterized as 6-hydroxytritriacontan-3-one[21]. Compound V, white granules, m.p. 83-84 ^{O}C and molecular formula $C_{30}H_{60}O_3$, [M^+,468] was arrived from its FAB mass spectrum. The important peaks observed in the IR spectrum were at 3390 (O-H stretch), 730 (C=O stretch), 1135 (O=C-O stretch) 725 and 710 cm^{-1} [doublet –$(CH_2)_n$ bending vibration where n > 4]. In the ^{1}H NMR spectrum, a set of two proton triplets appearing at δ_H 4.11 and δ_H 2.24 corresponded to the methylene groups attached to ester oxygen and ester carboxyl respectively. A multiplet centered at δ_H 3.55 appeared due to the proton attached to hydroxy bearing carbon. A triplet corresponding to six protons at δ_H 0.81 was indicative of two methyl groups and another set of triplet for methylene protons appeared at δ_H 1.18 and δ_H 7.4. In its mass spectrum molecular ion peak appeared at *m/z* 468 [M^+] and the spectrum showed significant peak at *m/z* 411, 383, 267, 201, 171, 85 and 57 along with many peaks with uniform loss of 14 mass units. The side chain nature was confirmed by the absence of an [M^+-15] ion[21], where as the presence of a peak corresponding to [M^+ + 1] ion was characteristic of its asymmetrical nature[22,23], evidenced the compound V as 6-hydroxy pentacosylpentanoate. Compound VI, white plates, m.p. 72-73 ^{O}C and molecular formula $C_{21}H_{42}O_2$ [M^+, 326] was arrived from its FAB mass spectrum. The important peaks observed in the infrared spectrum were at 1740 (C=O stretch), 1180 (O=C–O stretch), 730 and 720 cm^{-1} (doublet (-CH_2)$_n$– bending vibrations where n > 4). The molecular ion peak was observed at *m/z* 326. The base peak appeared at *m/z* 57 which could be assigned to CH_3–CH_2–CO– ion, other peaks were observed at *m/z* 297 [M^+–C_2H_5], 269 [M^+–C_2H_5–CO] and by loss of 14 mass units which suggests that the compound V to be ethyl nonadecanoate[24]. Compound VII gave positive test for flavonoid glycoside showed orange spot on TLC plate when developed with NP/PEG reagent and deep purple spot under UV light using 365 nm UV lamp and a yellow-green spot in presence of NH_3 vapours indicated the

presence of –OH group at C-5 and C-4´ in flavonoid[6]. UV spectrum in MeOH showed absorption at 346 nm, which is a characteristic absorption of orientin. On hydrolyzing with 5% HCl followed by extraction with $CHCl_3$ + MeOH (20%), gave luteolin and the aqueous layer when neutralized by adding $NaHCO_3$ solution afforded glucose. It was isolated earlier in our laboratory from *Opuntia dillenii*[25]. On the basis of above observations compound VII was characterized as orientin[6]. Compound VIII furnished positive test for flavonoid glycoside. The compound showed R_f 0.65 and when developed with NP/PEG reagent appeared as yellow spot under UV light at 365 nm. It showed a deep purple spot and a yellow-green spot in presence of ammonia vapours, thus indicating the presence of –OH group at C-5 and C-4´ in compound VIII. UV spectrum in methanol showed absorptions at 302 (sh) and 336 nm, which are characteristic for vitexin[6]. The identity was further cofirmed by co-TLC and co-IR with sample of vitexin isolated earlier from *Vitex negundo*[25].

Thus, eight compounds were obtained from the benzene fraction of defatted ethanol extract from the stem bark of *Parkinsonia aculeata.* Among the compounds, orientin and vitexin were the major coloured compounds. From the literature, it was known that both have antibacterial[26] and antifungal[27] properties. Woolen textile materials are considered as good media for generation and propagation of microbes and the sheep fabrics are used as cold protective fabrics requires such kind of protection to increase their value. Natural dyes are considered as eco-friendly dying source and if they may provide additional benefits as skin friendly or microbes protective, the value ability of fiber may be increased increases by many fold. Hence, in the present study, it was attempted to dye the sheep wool fibers with the orientin and vitexin which were isolated from the benzene extract of the stem bark of *Parkinsonia aculeata.*

Colourimetric value**:** Table 1 and 2 showed that the L* (Lightness- Darkness index), a*(yellow- Greeness index in case of orientin and brown- Greeness index in case of vitexin), and b* (Yellowness- Blueness index in case of orientin and browness- Blueness index in case of orientin) value of fabric dyed with orientin and vitexin, respectively. The orientin dyed the fabric were yellow in

colour whereas vitextin dyed fabrics were brown in colour with and without the use of metal salts (mordants). The lightness L* value of the samples were reduced after dyeing and mordanting. It showed that that the dye ability of the orientin and vitexin. In the case of mordanted samples, the formation of dye-metal coordinate complex generally reduces the lightness of dyed textiles with insignificant modification in the chrome of the shade. The a* value of the dyed samples showed yellow colour shade for orientin and brown for vitexin of the dyed fabric samples. There was no significant change in the chrome after mordanting except in the case of ferrous sulphate mordanted sample which showed slight increase in green tint in case of orientin. The b* values showed that the samples were yellow and brown in shades. All the mordanted samples were slightly bluer in shade. Overall, the orientin and vitexin dyed the fabric satisfactorily and produced corresponding yellow and brown shades with slight variation in the tones with the addition of different mordants.

Fastness properties: For washing fastness, the rating of 5 is excellent and 1 is very poor and for light fastness the rating of 8 is excellent and 1 is very poor[28].The fastness ratings of the orientin and vitexin dyed fabrics have been given in Table 1 and 2. The dyed fabric showed good washing and light fastness rating in the absence of mordants. The mordant treated samples were shown enhancement in the fastness properties due to the formation of coordination complex among dye, metal and fabric. On the basis of above observations, it is confirmed that the orientin and vitexin can be used to dye the sheep fabrics to develop yellow and brown shades with good fastness properties along with antimicrobial protection.

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