SHORT REPORT

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Dyeing Performance of Aqueous Extract and Flavanone Glycosides from the Flowers of *Butea monosperma* (Lam.) Kuntze

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Abstract: Aqueous extract and two flavanone glycosides named 5,7-dihydroxy-4'-methoxy flavanone-5-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**1**) and 5,5'-dihydroxy-4',7-dimethoxyflavanone-5,5'-di-O-*β*-D-glucopyranoside (**2**) obtained from the flowers of *Butea monosperma* were studied for evaluate their dyeing properties on cotton fibers. The stem bark of *Myrica esculenta* was used as natural mordant whereas $SnCl_2$ and $FeCl_3$ as synthetic mordants. The combination of dye with mordants showed interesting shades with excellent washing and light fastness properties.

Keywords: *Butea monosperma*; *Myrica esculenta*; Natural Dyes; Flavanone glycosides; Light fastness.

1. Plant Source

Butea monosperma (Lam.) Kuntze, vern. Dhak or Palas of Fabaceae family is a deciduous tree, distributed throughout India extending up to the North-West Himalaya. The flowers have been used to prepare a traditional yellow dye in India [1-2]. Herein, we report the dyeing properties of flowers and their constituents to validate the traditional use of the plant in dyeing while using *Myrica esculenta* (Myricaceae), an evergreen tree with brownish-grey bark, was used as natural mordant.

Fresh flowers of *B. monosperma* collected from Pauri Garhwal (Uttarakhand) during March 2011 were identified from Department of Botany, HNB Garhwal University, Srinagar and a voucher specimen (GUH-8387) of the plant is available in the departmental herbarium for future records.

2. Previous Studies

Flowers of *B. monosperma* have been used as blood purifier whereas seeds as antiseptic and antihelmentic in Indian traditional medicine [1]. Previous phytochemical reports on the plant have led to isolation of alkaloids [3,4], flavonoids [5,6], proanthocyanidins [7], terpenoids [8] and tannins [9].

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M. esculenta has been used as a traditional remedy for fever [1]. The leaf furnished flavonoids and steroids [10] whereas the stem bark used as natural mordant [11,12].

3. Present Study

Shade dried and crushed flowers (2 kg) of *B. monosperma* were successive extracted with n-hexane, chloroform and finally with methanol at 40-50 ºC in a soxhlet apparatus. The solvents were evaporated upto dryness under reduced pressure to yield 50, 80 and 150 g extracts, respectively. The methanolic extract was pre-adsorbed with silica gel (1:1) and then applied on the top of a column (15×100 cm) prepared by silica gel (Merck, 60-120 mesh, 400 g) in $CHCl_3$. Elution was first started with $CHCl_3$ and then with $CHCl_3$-MeOH by increasing quantity of MeOH upto 50%. The fractions obtained from column were collected every 100 ml and combined on the basis of TLC analysis. The elution of 18 and 20% MeOH in $CHCl_3$ afforded compound **1** and **2**, respectively (Fig. 1).

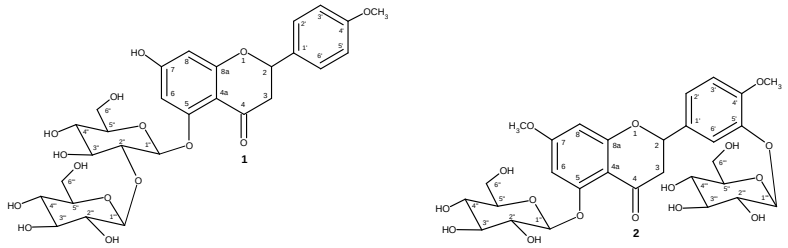

Figure 1. Chemical structures of **1** and **2**

*5,7-dihydroxy-4'-methoxyflavanone-5-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**):* A yellow hygroscopic solid (68 mg); mp: 205-208 ºC (incur.); UV λ_{max}^{MeOH}: 268, 322 nm; IR υ_{max}^{KBr}: 3340, 1645, 1500, 1392, 1368, 1010 cm^{-1}; FABMS (*m/z*, rel. abun.) 632.54 $[M+Na]^+$ (13%), 610.56 $[M]^+$ (9%), 447.28 $[M\text{-glu}]^+$ (32%), 285.27 $[M+H\text{-}2\times glu]^+$ (40%), 161.17 (53%), 151.42 (100%), 147.21 (19%), 91.35 (45%); Elemental analysis: (found C, 55.13; H, 5.58; O, 39.29%; calcd. for $C_{28}H_{34}O_{15}$: C, 55.08; H, 5.61; O, 39.31%); ^{1}H NMR: δ 5.39 dd (*J*=4.2, 13.2 Hz; H-2); 2.68 dd (trans, *J*=4.2, 12.5 Hz; H-3); 3.21 dd (cis, *J*=12.2, 16.4 Hz; H-3); 6.36 d (*J*=2.5 Hz; H-6); 6.96 d (*J*=2.5 Hz; H-8); 7.37 dd (*J*=2.5, 10.4 Hz; H-2'); 6.88 d (*J*=10.4 Hz; H-3'); 6.88 d (*J*=8.4 Hz; H-5'); 7.37 dd (*J*=2.5, 12.4 Hz; H-6'); 5.21 d (*J*=6.8 Hz; H-1"); 3.67 m (H-2"); 3.93 m (H-3"); 3.43 m (H-4"); 3.56 m (H-5"); 3.11 m (H-6"); 4.8 d (*J*=6.7 Hz; H-1'"); 3.96 m (H-2'"); 3.72 m (H-3'"); 3.43 m (H-4'"); 3.66 m (H-5'"); 3.16 m (H-6'"); 3.06 s (OMe-4'); 12.5 (OH-7) and ^{13}C NMR: δ 79.2 (C-2); 43.1 (C-3); 183.6 (C-4); 105.3 (C-4a); 163.4 (C-5); 99.5 (C-6); 158.5 (C-7); 97.7 (C-8); 156.9 (C-8a); 133.2 (C-1'); 115.1 (C-2'); 116.3 (C-3'); 145.7 (C-4'); 116.8 (C-5'); 115.6 (C-6'); 102.8 (C-1"); 77.6 (C-2"); 76.5 (C-3"); 69.3 (C-4"); 73.7 (C-5"); 60.8 (C-6"); 103.6 (C-1'"); 74.4 (C-2'"); 76.7 (C-3'"); 68.9 (C-4'"); 75.3 (C-5'"); 61.6 (C-6'"); 55.6 (OMe-4').

*5,5'-dihydroxy-4',7-dimethoxyflavanone-5,5'-di-O-β-D-glucopyranoside (**2**):* Yellow needles (54 mg), mp: 196-198 ºC (incur.); UV λ_{max}^{MeOH}: 230, 270, 314 nm; IR υ_{max}^{KBr}: 3325, 1665, 1480, 1402, 1352, 998 cm^{-1}; FABMS (*m/z*, rel. abun.) 641.59 $[M+H]^+$ (17%), 640.58 $[M]^+$ (8%), 477.44 $[M\text{-glu}]^+$ (32%), 315.30 $[M+H\text{-}2\times glu]^+$ (41%), 177.17 (23%), 165.13 (100%), 91.35 (42%); Elemental analysis: (found C, 54.31; H, 5.63; O, 39.97%; calcd. for $C_{29}H_{36}O_{16}$: C, 54.37; H, 5.66; O, 39.96%); ^{1}H NMR: δ 5.36 dd (*J*=4.2, 13.2 Hz; H-2); 2.65 dd (trans, *J*=4.2, 12.5 Hz; H-3); 3.23 dd (cis, *J*=12.2, 16.4Hz; H-3); 6.88 d (*J*=2.5 Hz; H-6); 6.98 d (*J*=2.5 Hz; H-8); 7.25 dd (*J*=2.5, 10.4 Hz; H-2'); 7.01 d (*J*=10.4 Hz; H-3'); 6.78 d (*J*=2.5 Hz; H-6'); 5.16 d (*J*=6.7 Hz; H-1"); 3.65 m (H-2"); 3.91 m (H-3"); 3.39 m (H-4"); 3.51 m (H-5"); 3.13 m (H-6"); 5.12 d (*J*=6.7 Hz; H-1'"); 3.89 m (H-2'"); 3.69 m (H-3'"); 3.38 m (H-4'"); 3.61 m (H-5'"); 3.14 m (H-6'"); 3.49 s (OMe-4'); 3.25 s (OMe-7) and ^{13}C NMR: δ 79.6 (C-2); 43.8 (C-3); 183.2 (C-4); 108.1 (C-4a); 164.0 (C-5); 99.8 (C-6); 159.0 (C-7); 97.4 (C-8); 157.1 (C-8a); 133.9 (C-1'); 116.1 (C-2'); 117.2 (C-3'); 148.5 (C-4'); 152.3 (C-5'); 118.4 (C-6'); 103.3 (C-1"); 73.8 (C-2"); 76.9 (C-3"); 70.2 (C-4"); 72.9 (C-5"); 63.2 (C-6"); 102.9 (C-1'"); 74.3 (C-2'"); 77.5 (C-3'"); 69.2 (C-4'"); 73.4 (C-5'"); 62.3 (C-6'"); 56.1 (OMe-4'); 55.9 (OMe-7).

Dyeing and mordanting: The dyeing process was carried out by following earlier researches [11-13] with some modifications. Briefly, fresh flowers of *B. monosperma* and bark of *M. esculenta* (500 g each) were crushed and extracted exhaustively in water up to 60° for 4h separately and the same were used for the study. The cotton threads and cloths were pretreated before dyeing by dipping them in soap solution and then washed with clean water thoroughly till completely free from detergent in order to remove dirt, dust or greases from the yarn. The extracted dye solution was divided into four parts and 100 ml of each was taken in separate dye baths. The 1st dye bath contains pure dye solution, whereas 25 ml of *M. esculenta* extracts, $SnCl_2$ and $FeCl_3$ (10 ml, 0.0015 gL^{-1} each) were added to 2nd, 3rd and 4th dye bath, respectively. The scoured, skinned presoaked cotton thread (1 g) and cotton cloth (6 cm^2) were dipped in each dye baths separately. These were heated upto 4 h with occasional stirring and allowed to cool at room temperature. Each sample was removed from the dye bath and dried in shade. The optical density of each left over solution was determined one by one using UV/Vis spectrometer and finally percentage absorption was calculated by formula [P.A. = {(O.D. before dyeing – O.D. after dyeing) ÷ O.D. before dyeing} × 100]. Each dyed sample was placed on a card-board frame along with blue standard rating. The dyed cotton thread and cloth samples were covered with a black object in such a manner that half of the samples exposed to light. Now the samples were placed inside the Fadometer to determine the rating for light fastness. On the other hand, the color fastness to washing was determined by dipping the dyed samples in the detergent solution for 30 min. and then rinsed in running water. After washing, the samples were shade dried and rating of color fastness was determined by Laundrometer.

Compound **1** was isolated as dark yellow hygroscopic solid, soluble in chloroform and recognised as flavanone glycoside by means of positive Gibbs, $FeCl_3$ and Molish tests. The molecular formula was deduced as $C_{28}H_{34}O_{15}$ from its molecular ion peak at *m/z* 610.5587 $[M]^+$ (calcd. for 610.5606) in its positive HR-FABMS. The important IR bands at 3340, 1645 and 1500 cm^{-1} were due to presence of chelated OH, C=O and C=C functions. The UV absorption at 268, 322 in MeOH; 277, 295 in NaOMe and 257sh, 277, 300 in $AlCl_3$ showed glycosidic nature of **1** which was further supported by two downfield signals for anomaric centers at δ 5.21 (d, *J*= 6.8 Hz) and 4.8 (d, *J*= 6.7 Hz) in the ^{1}H NMR, and 102.8 and 103.6 in the ^{13}C NMR. The coupling constant *J*= 6.8 and 6.7 Hz, respectively were indicative for β-D configuration of both sugars. The DEPT (135º) spectrum exhibited twenty eight signals corresponding to one methyl, three methylene, seventeen methine and seven quaternary carbons. The ^{1}H NMR spectrum displayed three ABX type signals at δ 5.39 (dd, *J*= 4.2 and 13.2 Hz) for H-2 with δ 2.68 (dd, *J*= 4.2 and 12.5 Hz) for H-3 trans, and δ 3.21 (dd, *J*= 12.2, 16.4 Hz) for H-3 cis, characteristic of flavanone [14]. In the ^{13}C NMR, three downfield signals at δ 145.7 (C-4'), 158.5 (C-7) and 163.4 (C-5) were suggestive to three oxygen substituted carbons including one methoxyl (δ 3.06 in ^{1}H and δ 55.6 in ^{13}C NMR), one hydroxyl (δ 12.5 in ^{1}H NMR) and obviously one glycosidic substituted. The linkage of substitution was confirmed by means of COSY, NOESY and HMBC correlations (Fig. 2). The long range correlation of δ 12.5 (OH) to 97.7 (C-8)/ 99.5 (C-6)/ 158.5 (C-7) in the HMBC confirmed the position of hydroxyl group at C-7. Similarly, the linkage of methoxyl group at C-4' was confirmed by the NOESY correlation of δ 3.06 (OCH_3) to 6.88 (H-3', 5'), and the HMBC of δ 55.6 (OCH_3) to 145.7 (C-4'). An important $^3J_{CH}$ correlation of δ 5.21 (H-1") to 163.4 (C-5) in the HMBC confirmed the linkage of glycosidic part at C-5. The ^{13}C NMR value of a sugar carbon at δ 77.6 (C-2") (downfield shift of ~5 ppm) showed that C-2" was the linkage to another sugar unit. The hydrolysis (12% MeOH-HCl, 10 ml, 60-80 ºC, 8 h) as well as methanolysis (first methylation and then hydrolysis) of **1** followed by paper chromatography confirmed the presence of two glucose molecules with 1→2 linkage. [15]. The mass spectrum was found very informative in respect of structure elucidation in which the fragments at *m/z* 447.28 and 285.27 were corresponding to the loss of one and two glucosyl, respectively from molecular ion. The other fragments at *m/z* 151.42 (100%), 161.17, 147.21 and 91.35 were typical for a flavanone [16]. Hence, the structure of **1** was elucidated as 5,7-dihydroxy-4'-methoxyflavanone-5-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

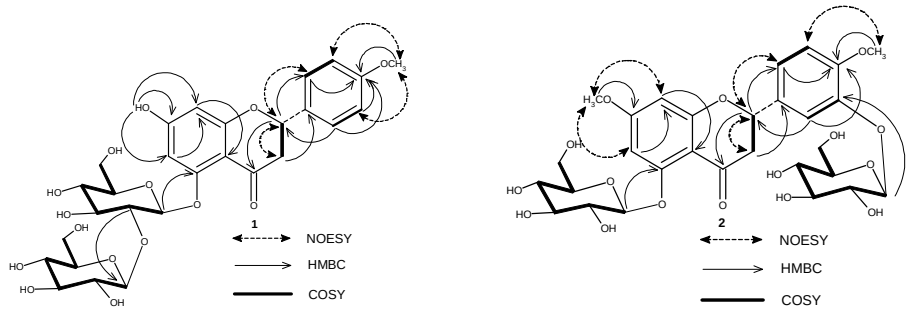

Figure 2. Selected 2D NMR correlations

Compound **2**, obtained as yellow needles, deduced the molecular formula $C_{29}H_{36}O_{16}$ from quasi molecular ion at *m/z* 641.5901 $[M+H]^+$ and molecular ion at *m/z* 640.5802 (calcd. 640.5865) in its positive HRFABMS. It was also recognised as flavanone glycoside by means of characteristic chemical tests and by the IR and UV spectra, which were found somewhat similar to that of **1**. The DEPT (135º) spectrum showed twenty nine signals refer to two methyl, three methylene, sixteen methine and eight quaternary carbons. Four downfield signals in the ^{13}C NMR at δ 148.5 (C-4'), 164.0 (C-5), 152.3 (C-5') and 159.0 (C-7) were corroborated to four oxygen substituted carbons. Among them, two signals were assigned for methoxyl functions (δ 55.9 and 56.1). Two anomeric signals at δ 5.12 and 5.16 (both doublet with J= 6.7 Hz) in the ^1H NMR and δ 102.9 and 103.3 in the ^{13}C NMR were clearly showed the presence of two sugar moieties. The partial hydrolysis as well as methanolysis, resulted two glucose molecules linked to two different carbons. The positions of all substitutions were confirmed by HMBC, NOESY and COSY studies (Fig. 2). The NOESY correlation of δ 3.49 (OCH_3-4') to 7.01 (H-3'); 3.25 (OCH_3-7) to 6.88 (H-6)/ 6.98 (H-8) and the HMBC of 5.16 (H-1") to 164.0 (C-5); 5.12 (H-1"') to 152.3 (C-5'); 3.49 to 148.5 (C-4'); 3.25 to 159.0 (C-7) confirmed the linkage of methoxyl groups at C-4' and C-7 whereas glucose molecules at C-5 and C-5'. The mass spectrum exhibited a molecular ion at *m/z* 640.58 which produced two daughter ions at 477.44 and 315.30 due to loss of two glucosyl moieties. The fragmentation pattern of **2** was found similar to that of **1**, characteristic for a flavanone. On the basis of above discussion, the compound **2** was characterised as 5,5'-dihydroxy-4',7-dimethoxyflavanone-5,5'-di-O-β-D-glucopyranoside.

Aqueous flower extract showed UV absorption at 320 nm whereas **1** and **2** showed at 322 and 314 nm, respectively (characteristic for flavonoids) [17]. The extract, **1** and **2** showed remarkable absorption percentage by 41.77, 51.14 and 49.89, respectively. The extract with natural mordant (*M. esculenta*) exhibited better absorption (47.93%) as compared to that of synthetic (Table 1).

Table 1. Percentage absorption of dyes and their fastness properties.

Dye	Mordant	λ_{max}^{MeOH}	Percentage absorption	Color imparted	Light fastness	Washing fastness
BM	-	320	41.77	Gold2 #EEC900	4-5	4-5
	ME	360	47.93	Lightsalmon3 #CD8162	5-6	5-6
	$FeCl_3$	320	25.72	Darkgoldenrod #B8860B	3-4	3-4
	$SnCl_2$	320	27.04	Gold3 #CDAD00	2-3	3-4
1	-	322	51.14	Yellow #FFFF00	3-4	4-5
2	-	314	49.89	Gold FF700	4-5	3-4

ME =Myrica esculenta BM = Butea monosperma

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