Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2011, 3 (3):350-357

(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Natural Dye constituents from rind of *Punica granatum* and its application on Pashmina fabrics

Chhagan Lal[1], Mahesh C. Sharma[1], Dinesh B. Shakyawar[2*], A. S. M. Raja[2], Kuldeep K. Sharma[1] and Pawan K. Pareek[2]

[1]*Centre of Advanced Studies, Department of Chemistry, University of Rajasthan, Jaipur, India*
[2]*Central Sheep and Wool Research Institute, Avikanagar (Rajasthan),* India

---

ABSTRACT

The chloroform fraction of the aqueous extract of the rind of Punica granatum on its chemical investigation afforded seven already reported compounds viz.1-O-Isopentyl-3-O-octadec-2-enoyl glycerol (I), 1-O-trans,cis,trans-9,11,13-octadecatrienoyl glycerol (II), β-Sitosterol laurate (III), β-Sitosterol myristate (IV), Punicanolic Acid (V), Luteolin (VI) and Tricetin (VII). The structures of isolated compounds were ascertained using various spectral (IR, ^{1}H, ^{13}C NMR, MS) techniques. Compounds I, II, VI and VII were found to be couloured in nature and tested as dye substances on Cashmere (Pashmina) wool and showed promising dyeing properties.

Keywords: Pomegranate, Natural dye, Cashmere (Pashmina) wool.

INTRODUCTION

Pomegranate *(Punica granatum)* is an ancient fruit with great medicinal importance related to punicaceae family [1,2]. Pomegranate is a high value crop and cultivated throughout India. Entire tree of pomegranate is of great economic importance. Apart from its demand for fresh fruits and juice, the processed products like wine and candy are also gaining importance in world trade. All parts of pomegranate tree have great therapeutic value and use in leather and dying industry. It is an ideal crop for the sustainability of small holdings, as pomegranate is well suited to the topography and agro-climate of arid and semi-arid regions [3]. It is useful to cure diarrhoea and also has anthelmintic and vermifuge properties [4, 5]. The earlier studies of *P. granatum* flowers have shown the presence of sterols, phenolic compounds, and pentacyclic triterpenes [6, 7].

In recent decades, an increasing propensity towards the use of natural substances instead of the synthetic ones has been observed. As the synthetic materials and products are more complex instead of natural substances, it will take a long instant for decomposing and return to nature; thus causing a lot of environmental pollution. Also with the increase in the price of raw

materials, the problem of cost benefits for chemical production is becoming more considerable. Natural dyes, such as tannin compounds, used as natural dyes, are gaining importance, due to their benefits for human health.

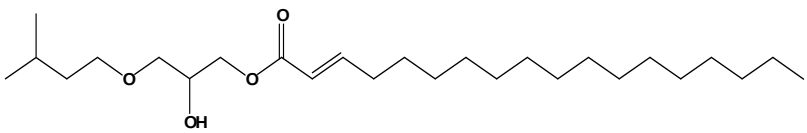
Looking at the importance of this plant, it is thought of interest to accommodate the isolation and characterization of chemical constitution rind of pomegranate regarding their application for dyeing of Cashmere (Pashmina) Shawl.

MATERIALS AND METHODS

IR spectra were recorded on FT-IR 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. Qualitative and quantitative TLC were conducted on aluminium sheets Kieselgel $60F_{254}$ (E.Merck). Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus and are uncorrected.

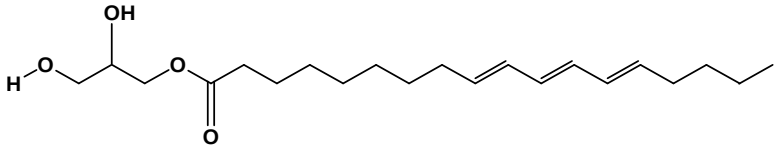
The rinds of *P. Granatum* were collected from nearby Jaipur, semi-arid region of Rajasthan. The air dried and coarsely powdered rind (2 Kg) was soaked in 5 litres of water and left for overnight. Then, it was boiled for 1 hr to get a water soluble dye solution. The extract was filtered and concentrated under reduced pressure and the resulting reddish brown solution was separated into chloroform and ethyl acetate fractions using a separating funnel. On analytical TLC examination the $CHCl_3$ segment showed presence of 4 coloured compounds, which was finally observed with the presence of 7 compounds after spraying with ceric ammonium sulphate in 2*N* H_2SO_4 solution. The Brownish orange coloured semisolid residue as $CHCl_3$ extract (30 g) was subjected to column chromatography over silica gel and collected 7 fractions. Fraction eluted with pet-ether (100%) gave compound-I as yellow oily mass, 60 mg. Fraction eluted with pet ether-chlorofrom (4:1) also afforded yellow oily mass, 110 mg compound II while fraction eluted with pet ether - chloroform (3:2) gave colourless fibrils, 90 mg as compound-III. Fraction eluted with pet-ether-chloroform (2:3) and followed by prep TLC gave colourless flakes, 30 mg as compound-IV. Fraction obtained after eluting with pet-ether- chloroform (1:2) gave white amorphous powder, 60 mg as compound-V. Fraction eluted by chloroform (100%) afforded compound VI as yellow needles. Compound VII yielded as orange prisms, 80 mg eluted by chloroform: ethyl acetate (4:1). The ethylacetate and aqueous layer will be analysed in near future.

1-*O*-Isopentyl-3-*O*-octadec-2-enoyl glycerol (I). Yellow oily mass, IR (KBr, cm^{-1}): 3415, 2970, 1618; ^{1}H NMR (Solvent $CDCl_3$; 300 MHz; δ ppm): 0.96 (t, 3H, J 3.3), 1.01 (d, 6H, J 6.1), 1.29 (m, 22H), 1.33 (m, 2H), 1.42 (m, 2H), 1.83 (m, 1H), 1.96 (m, 2H), 2.0 (s, OH), 3.27 (t, 2H, J 4.2), 3.52 (d, 2H, J 1.1), 4.14 (dt, 2H, J 2.2), 4.30 (d, 1H, J 1.8), 5.83 (d, 1H, J 1.1), 6.88 (m, 1H); ^{13}C NMR (Solvent $CDCl_3$; 75.45 MHz; δ ppm): 14.0 (CH_3), 22.3 (2 × CH_3), 23.1 (CH_2), 24.8 (CH), 30.0 (2 × CH_2), 30.3 (7 × CH_2), 30.4 (2 × CH_2), 32.5 (CH_2), 32.7 (CH_2), 40.1 (CH_2), 67.7 (CH_2), 70.7 (CH_2), 72.3 (CH), 74.0 (CH_2), 147.6 (CH), 165.0 (C=O); MS *m/z* [M^{+}] 426, $C_{26}H_{50}O_4$.



(I)

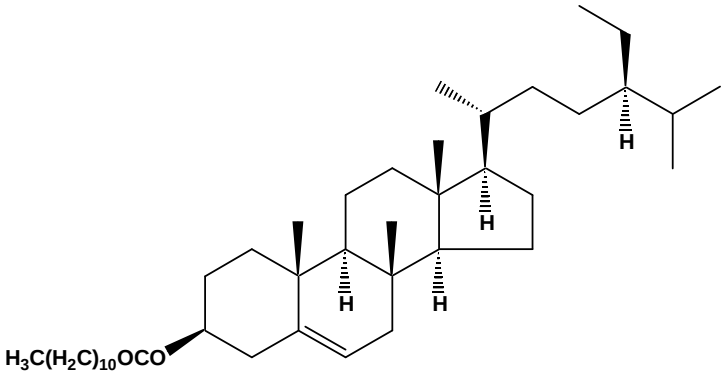
1-*O*-*Trans, cis, trans*-9,11,13-octadecatrienoyl glycerol (II). Yellow oily mass, IR (KBr, cm^{-1}): 3240, 2884, 2768, 1710, 780; ^{1}H NMR (Solvent $CDCl_3$; 300 MHz; δ ppm): 0.96 (t, 3H, J 7.8), 1.29 (m, 8H), 1.33 (m, 2H), 1.68 (m, 2H), 1.96 (m, 2H), 2.01 (s, OH), 2.25 (t, 2H, J 6.9), 3.68 (dd, 2H, J 11.6, 3.9), 3.90 (m, 1H), 4.23 (dd, 2H, J 11.9, 5.8), 5.42 (ddd, 1H, J 18.1, 9.8, 7.1), 5.72 (dd, 1H, 9.1, 5.4), 6.02 (dd, 1H, J 18.4, 6.0), 6.27 (dd, 1H J 10.0, 5.2), 6.51 (dd, 1H, J 10.0, 5.2); ^{13}C NMR (Solvent $CDCl_3$; 75.45 MHz; δ ppm): 13.90 (CH_3), 22.27 (CH_2), 25.1 (CH_2), 27.8 (CH_2), 28.02 (CH_2), 29.26 (CH_2), 29.28 (CH_2), 30.1 (CH_2), 32.5 (CH_2), 35.11 (CH_2), 63.80 (CH_2), 62.15 (CH_2), 71.20 (CH), 126.9 (CH), 128.3 (CH), 129.9 (CH), 131.95 (CH), 133.4 (CH), 174.1 (C=O); MS *m/z* [M^+] 391, $C_{21}H_{36}O_4$.



(II)

***β*-Sitosterol laurate (III)** colorless fibrils, m.p. 106–107^0 C; IR ν_{max} (KBr): 2880, 2820, 1740, 1615, 730; ^{1}H NMR (Solvent $CDCl_3$; 300 MHz; δ ppm): 0.68 (brs, 3H), 0.80 (d, 3H, J 3.6), 0.82 (d, 3H, J 6.1), 0.87 (t, 3H□, J 5.9), 0.90 (d, 3H, J 6.4), 0.94 (d, 3H, J 6.1), 1.10 (brs, 3H), 1.17 (m, 2H), 1.19 (m, 2H), 1.26 (brs, 2H), 1.28 (brs, 14H□), 1.38 (m, 2H), 1.43 (m, 1H), 1.49 (m, 1H), 1.56 (m, 7H), 1.70 (m, 3H), 1.78 (m, 2H), 1.85 (m, 2H), 2.15 (m, 3H), 2.62 (d, 2H, J 5.6), 2.19 (m, 2H□), 2.39 (m, 2H□), 2.56 (d, 2H□, J 5.8), 4.3 (m, 1H), 5.41 (m, 1H); ^{13}C NMR (Solvent $CDCl_3$; 75.45 MHz; δ ppm): 12.2 (2×CH_3), 14.1 (CH_3), 17.1 (CH_3), 17.9 (CH_3), 18.8 (CH_3), 19.9 (CH_3), 20.8 (CH_2), 23.5 (CH_2), 23.9 (CH_2), 24.8 (CH_2), 26.0 (CH), 27.6 (CH_2), 27.9 (CH_2), 28.2 (CH_2), 30.0 (2×CH_2), 31.5 (CH_2), 35.2 (CH_2), 37.4 (C), 38.1 (CH_2), 39.8 (CH_2), 41.1 (CH_2), 44.7 (CH), 47.7 (CH), 54.2 (CH), 55.9 (CH), 71.0 (COH), 121.2 (CH), 139.8 (C), 174.1 (C=O), MS *m/z*: 596 $[M]^+$ ($C_{41}H_{72}O_2$).

Alkaline hydrolysis of *β*-sitosterol laurate:
100 mg of compound – III was refluxed with methanolic KOH at 100 ^{O}C over boiling bath for 1 hour. After cooling, the solution was taken in distilled water and partitioned with diethyl ether. The ether soluble fraction was dried over anhydrous Na_2SO_4, then ether was removed under reduced pressure yielded β-sitosterol (50 mg, m.p. 136-138 ^{0}C). The water soluble fraction was acidified with dil HCl and partitioned with diethyl ether afforded lauric acid (20 mg, m.p. 40-41 ^{0}C).

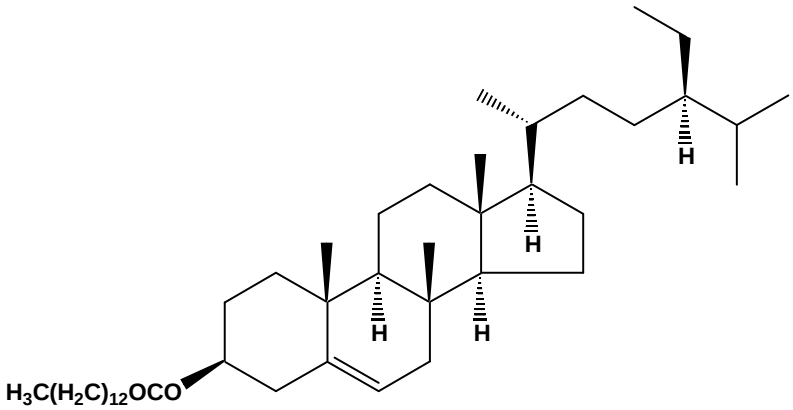


(III)

***β*-Sitosterol myristate (IV)** colorless flakes, m.p. 116–117 ^{0}C; IR ν_{max} (KBr): 1744, 1628, 733 cm^{-1}; ^{1}H NMR (Solvent $CDCl_3$; 300 MHz; δ ppm): 0.70 (brs, 3H), 0.78 (d, 3H, J 6.0), 0.83 (d, 3H, J 4.0), 0.82 (t, 3H□, J 5.9), 0.90 (d, 3H, J 6.6), 0.98 (d, 3H, J 6.6), 1.10 (brs, 3H), 1.21 (m,

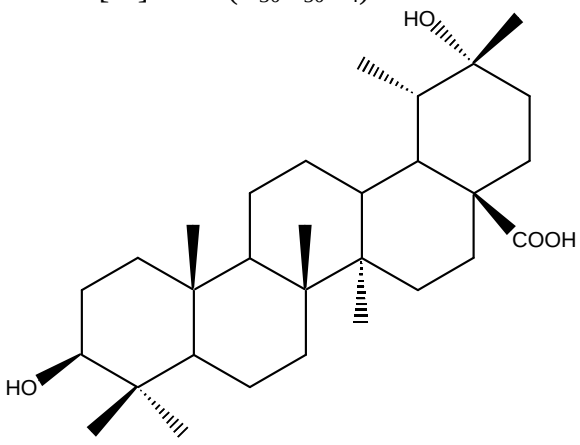
2H), 1.24 (m, 2H), 1.28 (brs, 18H□), 1.32 (brs, 2H), 1.40 (m, 2H), 1.45 (m, 1H), 1.52 (m, 1H), 1.60 (m, 7H), 1.72 (m, 3H), 1.84 (m, 2H), 1.90 (m, 2H), 2.12 (m, 3H), 2.21 (m, 4H), 2.32 (m, 2H□), 2.74 (d, 2H□, J 5.8), 4.25 (m, 1H), 5.56 (m, 1H); ^{13}C NMR (Solvent $CDCl_3$; 75.45 MHz; δ ppm): 12.6 (2×CH_3), 16.1 (CH_3), 17.9 (CH_3), 18.7 (CH_3), 18.6 (CH_3), 19.5 (CH_3), 21.1 (CH_2), 22.3 (CH_2), 23.6 (CH_2), 24.9 (CH_2), 26.1 (CH), 27.8 (CH_2), 28.0 (CH_2), 30.2 (4×CH_2), 30.4 (CH_2), 32.4 (CH_2), 35.9 (2×CH_2), 37.2 (C), 38.1 (CH_2), 39.6 (CH_2), 42.6 (2×CH_2), 43.9 (CH), 52.0 (CH), 56.1 (CH), 57.1 (CH), 64.1 (CH), 65.5 (CH_2), 121.0 (CH), 139.8 (CH_2), 174.4 (C=O), MS *m/z:* 624 $[M]^+$ ($C_{43}H_{76}O_2$).

Alkaline hydrolysis of *β*-sitosterol myristate:
100 mg of compound – IV was refluxed with methanolic KOH at 100 ^{O}C over boiling bath for 1 hour. After cooling the solution was taken in distilled water and partitioned with diethyl ether. The ether soluble fraction was dried over anhydrous Na_2SO_4 and the ether was removed under reduced pressure yielded *β*-sitosterol (62 mg, m.p. 136-138 ^{0}C). The water soluble fraction was acidified with dil HCl and partitioned with afforded myristic acid (21 mg, m.p. 55-56 ^{0}C).



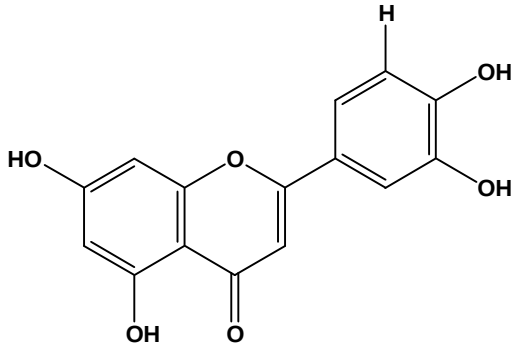
(IV)

Punicanolic Acid (V) white amorphous powder, m.p. 276-278 °C, IR ν_{max} (KBr): 3380 (-OH) and 1730 (C=O) cm^{-1}, ^{1}H NMR (Solvent $CDCl_3$; 300 MHz; δ ppm): 0.88 (s, 3H), 1.04 (s, 3H), 1.08 (s, 3H), 1.14 (s, 3H), 1.18 (s, 3H), 1.39 (s, 3H), 1.44 (d, 3H, J 6.2), 2.11 (s, OH), 3.62 (dd, 1H, J 6.2, 11.0), 11.5 (brs, chelated H). ^{13}C NMR (Solvent $CDCl_3$; 75.45 MHz; δ ppm): 14.9, 16.2, 17.0, 17.2, 20.1, 29.1, 31.2 (7×CH_3), 35.5 (10×CH_2), 74.1 (quaternary carbon), 78.2 (5×CH), 181.1 (COOH); MS *m/z* $[M]^+$ 474 ($C_{30}H_{50}O_4$).



(V)

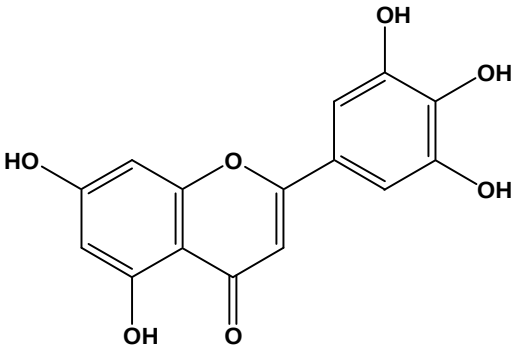
Luteolin (VI) Yellow needles; m.p. 322 ^{0}C, ^{1}H NMR (Solvent $CDCl_3$; 300 MHz; δ ppm): 6.54s (1H), 6.40 d (1H, J= 1.9Hz), 6.70 d (1H, J = 1.9 Hz), 12.36 brs (OH), 6.82s (2H), 6.12 (1H); ^{13}C NMR (Solvent $CDCl_3$; 75.45 MHz; δ ppm): 167.2, 107.8 (=CH-), 186.7 (=C=O), 163.6, 101.1, 165.4, 97.9 (=CH-), 154.1, 106.2, 124.6, 105.9 (=CH-), 153.2, 141.2, 151.1, 104.2; MS *m/z* $[M]^+$ 286 ($C_{15}H_{10}O_6$).



2-(3,4-Dihydroxy-phenyl)-5,7-dihydroxy-chromen-4-one

(VI)

Tricetin (VII) Orange prisms, m.p. 170 ^{0}C, IR ν_{max} (cm^{-1}): 3415, 1610; ^{1}H NMR (Solvent $CDCl_3$; 300 MHz; δ ppm): 6.57s (1H), 6.36 d (1H, J= 1.8Hz), 6.72d (1H, J = 1.8 Hz), 12.4 brs (OH), 6.84s (2H), ^{13}C NMR (Solvent $CDCl_3$; 75.45 MHz; δ ppm): 168.1, 108.1 (=CH-), 188.1 (=C=O), 164.1, 100.2, 166.1, 98.7(=CH-), 153.0, 107.4, 123.6, 108.01(=CH-), 154.2, 140.1, 152.1, 105.8 MS *m/z* $[M]^+$ 302 ($C_{15}H_{10}O_7$).



(VII)

Dyeing

Fabric weight	- X g
Natural dye extract	- X ml
Acetic acid	- 1%
MLR	- 1:50
Temperature	- at boil
Time	- 1-2 hrs
pH	- acetic

Required quantity of water was taken into dyeing vessel, added calculated amount of natural dye extract, and the extract thoroughly mixed. Added 1% acetic acid on the weight of the fabric and mixed thoroughly. Introduced the Cashmere (Pashmina) fabric into the dye bath at room temperature and allowed the fabric to completely wet. The temperature of the bath was increased slowly to boiling condition and stirred the fabric inside dye bath frequently. The duration of the dyeing was kept for 1 hr. After one hr, heating the bath was stopped and allowed cooling the dye

bath to room temperature. Removed the fabric from dye bath and washed the dyed fabric with clean water 2 times.

Mordanting

Fabric weight	- Xg
MLR	- 1:50
Ferrous sulphate	-5%
Acetic acid	- 1%
Temperature	- Room temperature
Time	- 12 hrs/over night

Required quantity of water was taken in the mordanting vessel, added calculated amount of aluminium sulphate and mixed the contents thoroughly. Added 1% acetic acid on the weight of the fabric and mixed thoroughly. Introduced the dyed Cashmere (Pashmina) fabric into the mordant solution at room temperature and allowed the fabric to completely wet. It was ensured that the fabric was completely dipped in water. The bath was kept for 12 hrs/overnight with occasional stirring. After 12 hrs, the bath was heated to 60-70°C for 1 hr. Then heating was stopped and then cooled the bath to room temperature. The dyed fabric was washed with water 2 times. For scraping excess dye from the fabric, the fabric washed with 0.5 % of detergent and finally it was again washed with clean water and dried.

RESULTS AND DISCUSSION

Compound I and II were isolated as yellow oily masses, their spectral evidences showed that compound I to be 1,3 di substituted glycerol and the compound II appeared as glycerol ester and found to be 1-O-Isopentyl-3-*O*-octadec-2-enoyl glycerol and 1-*O*-*trans*,*cis*,*trans*-9,11,13-octadecatrienoyl glycerol, respectively [8].

Compound III was obtained from petroleum ether–chloroform (3:2) eluants as a colorless fibrils, gave positive Liebermann – Burchard test [9] and Nollers tests [10] which confirms its steroidal nature and positive TNM test for the confirmation of unsaturation. Its IR spectrum displayed characteristic absorption bands for the ester group (1740 cm^{-1}), C=C stretching (1615 cm^{-1}), and long aliphatic chain (730 cm^{-1}). Analysis of its ^{1}H NMR spectrum revealed with two broad siglets at δ 0.68 and 1.10 in favour of C-18 and C-19 methyl protons, six doublets, first four at δ 0.94 (J = 6.1Hz), δ 0.91 (J = 6.4Hz), δ 0.82 (J = 6.1Hz), and δ 0.85 (J = 4.0Hz), accounted for C-21, C-26, and C-27 and C-29 methyl protons, remaining two doublets at δ 2.48 (J = 5.3Hz) and δ 2.51 (J = 5.4Hz) were ascribed to the C-20 methylene protons, a triplet at δ 0.87 (J = 5.9Hz) was due to terminal methyl protons, two broad multiplets at δ 5.23 and 4.18 attributed to the vinylic protons and C-3 carbinol proton, respectively. The ^{13}C NMR spectrum prominent peaks for the carbinol carbon at δ 70.1 (C-3), the vinylic carbon at δ 139.8 (C-5) and δ 120.1 (C-6), ester carbon at δ 174.1 (C-10), the primary methyl carbon at δ 14.1 (C-120), Its molecular ion peak was observed at m/z 596 consistent with the molecular formula $C_{41}H_{72}O_2$. Its identity was further confirmed by its alkaline hydrolysis yielded β-sitosterol and lauric acid. On the basis of the spectral data analysis and chemical reactions, the structure of III has been established as stigmasterol laurate [11].

Compound IV, was afforded by the purification of the fraction eluted from petroleum ether–chloroform (2:3) as a colorless needles. It furnished positive steroidal tests. Its spectral assignments showed that it is a steroidal ester having close resemblance with those reported for compound III, this ambiguity was diminished by its alkaline hydrolysis and the products such

obtained showed well comparable spectral and chemical properties as those reported for β-sitosterol and myristic acid, thus on the basis of intense literature survey the compound was designated as β-sitosterol myristate [11].

Compound V, the fraction eluted from pet. ether :chloroform (1:2) yielded colourless fibres (m.p. 276-278) after purification. Its IR spectrum revealed with the absorption peaks at 3380 (-OH) and 1730 (C=O) cm^{-1}. The ^{1}H NMR spectrum exhibited six singlets as δ 0.88, 1.04, 1.08, 1.10, 1.18, 1.39 and a doublet at δ 1.44 which were credited to seven methyl groups , their corresponding ^{13}C NMR signals were observed at δ 17.0, 16.2, 14.9, 17.2, 29.1, 31.2 and 20.1, respectively. The carboxyl carbon resonated at δ 181.1 and the quaternary carbon directly attached to it was observed at δ 73.0 in its ^{13}C NMR spectrum. Spectral analysis of this compound showed good agreement as those reported for Punicanolic Acid [12].

Fraction VI eluted by chloroform gave yellow needles on its final purification. Its ^{1}H NMR signals observed with a broad singlet at δ 12.88 ascribable for chelated hydroxyl groups. The rest were, one singlet at δ 6.72, four doublets at δ 6.20 (J =2.2 Hz), 6.36 (J = 1.6 Hz), 6.92 (J = 8.4 Hz) and 7.38 (J = 1.8 Hz,) and a double doublets at δ 7.45 (J=8.5, 1.8 Hz) were accountable for six methine protons, on the basis of spectral study the compound seemed to be luteolin [13].

Compound VII formed as orange prisms after separation from the fraction VII eluted by chloroform: ethyl acetate (4:1). ^{1}H NMR analysis observed with a broad singlet at δ 12.86 credited to hydroxy protons and a singlet at δ 6.8 resonated for H-2' and H-6'. Two doublets at δ 6.21 (2.2 Hz) and 6.38 (1.5 Hz) observed for H-6 and H-8 protons, hence the structure of the compound look alike to tricetin [14].

The rinds of *P. Granatum* dyed the Cashmere (Pashmina) wool acceptably with and without the use of mordants. The rinds of *P. Granatum* contain some colour compounds such as 1-O-Isopentyl-3-O-octadec-2-enoyl glycerol, 1-O-Trans,cis,trans-9,11,13-octadecatrienoyl glycerol, Luteolin and Tricetin. The colour obtained on the Cashmere (Pashmina) fabric using *P. Granatum* dye extract is given **Table 1**.

Table 1: Colours and washing fastness properties of Cashmere (Pashmina) shawl dyed with *P. Granatum* rinds.

S.No	Particulars	Colour obtained	Washing Fastness
1	*P. Granatum*	Pale yellow	4
2	*P. Granatum* + Aluminium sulphate	Bright Yellow	4-5
3	*P. Granatum* + Stannous chloride	Orange	4-5
4	*P. Granatum* + Ferrous sulphate	Dull green	4

It is observed that the *P. Granatum* extract dyed the Cashmere (Pashmina) wool with bright colours having good fastness. The washing fastness results showed that all the dyed samples of *P. Granatum* rinds extract have well to very good fastness properties on Cashmere (Pashmina) fabric with and without the use of mordants.

CONCLUSION

The spectra analysis of the extracted solid of pomegranate showed the presence of 1-O-Isopentyl-3-O-octadec-2-enoyl glycerol, 1-O-Trans,cis,trans-9,11,13-octadecatrienoyl glycerol, Luteolin and Tricetin. These compounds are responsible for coloring ability of textiles. The Cashmere (Pashmina) wool dyed with the extract of pomegranate rind showed bright colours

with good fastness properties. Hence, this process can be considered as a commercially viable process of dyeing Cashmere (Pashmina) fabrics. The process enhanced the value of *P. Granatum* as well as Cashmere (Pashmina) wool fabric due to waste utilization and eco friendly dyeing.

REFERANCES

[1] Anonymous, The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, (Publication and Information Directorate (CSIR), New Delhi, **2003**), 5, 425.
[2] N.D. Prajapati, S.S. Purohit, A.K. Sharma, T. Kumar, A Handbook of Medicinal Plants (Agrobios India, Jodhpur, **2003**), 432.
[3] http://www.nrcpomegranate.org/mandate.htm
[4] K.R. Kirtikar, B.D. Basu, Indian Medicinal Plants (Sri Satguru Publications, New Delhi, **2000**), 4, 1508.
[5] S.A.H. Naqvi, M.S.Y. Khan, S.B. Vohora, *Fitoterapia*, **1991**, 62, 221.
[6] T.H.W. Huang, Q.L. Yang, M. Harada, G.Q. Li, J.Yamahara, B.D. Roufogalis, Y.H. Li, *J. Cardiovasc. Pharmacol.* **2005**, 46, 856.
[7] E.P. Lansky, R.A. Newman, *J. Ethnopharmacol.* **2007**, 109, 177.
[8] M. O. Fatope, S. Khamis, S. A. Burtomani, Y. Takeda, *J. Agric. Food Chem.* **2002**, 50, 357.
[9] C. Liebermann, *Ber.* **1885**, 18, 1804.
[10] C. R. Noller, R. A. Smith, G. H. Harris, J. W. Walker, *J. Am. Chem. Soc.*, **1942**, 64 (12), 3047.
[11] P. Bagri, M. Ali, S. Sultana, V. Aeri, *J. Asian Natu. Produ. Res.*, **2009**, 11 (8), 710.
[12] Y. Xie, T. Morikawa, K. Ninomiya, K. Imura, O. Muraoka, Dan YUAN and M. Yoshikawa, *Chem. Pharm. Bull.*, **2008**, 56(11) 1628.
[13] J. H. Kwak, M. W. Kang, J. H. Roh, S. U. Choi, O. P. Zee, *Arch. Pharm. Res.*, **2009**, 32 (12), 1681.
[14] K. R. Markham, B. Ternai, R. Stanley, H. Geiger, T. J. Mabry, *Tetrahedron*, **1978**, 34, 1389.