repeating the experiment three times at both the levels. The average percentage recoveries of three brands are (99.10, 99.41, and 99.21) is also satisfactory indicating the good accuracy of the method (Table 2).

Now a day’s most of the ayurvedic formulations are lacking in defined quality control parameters. FDA has made the quality control and GMP mandatory for ayurvedic formulation, which has been implemented from 1st January 2003. Hence, now these preparations have to be tested for the identity, purity, potency, safety and efficacy so that they would gain universal acceptance. In the light of the above, the present study can be used as one of the parameters for standardization during the routine quality control of Ayurvedic eye drops.

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Phytochemical Examination of Prosopis cineraria L. (Druce) Leaves

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The phytochemical studies on the leaves of Prosopis cineraria resulted in isolation of methyl docosanoate, diisopropyl-9,10-dihydroxyicosane-1,20-dioate, tricosan-1-ol and 7,24-tirucalladien-3-one. While diisopropyl-10,11-dihydroxyicosane-1,20-dioate is a hitherto unreported compound, methyl docosanoate, tricosan-1-ol and 7,24-tirucalladien-3-one are being reported for the first time from P. cineraria. These compounds have been characterized on the basis of spectral and other data.

Prosopis cineraria (L.) Druce (Syn. P. spicigera L.) (fam: Leguminosae, subfam: Mimosaceae) is prickly tree or shrub and commonly found in dry and arid regions of north-western India, southern India, Pakistan, Afghanistan, Iran and Arabia. Leaves and pods are extensively used as fodder for cattle, camels and goats.

Prosopis species have also been extensively used in indigenous system of medicine as folk remedy for various ailments like leprosy, dysentery, bronchitis, asthma, leucoderma, piles, muscular tremors and wandering of the mind. It is also known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties. Leaf paste of P. cineraria is applied on boils and blisters, including mouth ulcers in livestock and leaf infusion on open sores on the skin. The smoke of the leaves is considered good for eye troubles. Jewers et al. have studied the

Table 2: Estimation of Tannin Content of Herbal Eye Drop Preparations and Recovery Studies

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tannin content (µg/ml)*±SD</th>
<th>Tannin added µg/ml</th>
<th>% Recovery</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand -A (Eye tone, Himayala)</td>
<td>420.00±1.391</td>
<td>100</td>
<td>99.10</td>
<td>0.084</td>
</tr>
<tr>
<td>Brand -B (Itis, Dey's Medical)</td>
<td>918.00±2.639</td>
<td>100</td>
<td>99.41</td>
<td>0.067</td>
</tr>
<tr>
<td>Brand -C (Opticare, Ozone Ayurvedics)</td>
<td>270.49±0.782</td>
<td>100</td>
<td>99.21</td>
<td>0.071</td>
</tr>
</tbody>
</table>

*Mean±SD of three determinations

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phytochemicals in the leaves of *P. cineraria* and reported alkaloid namely spicigerine; steroids namely campesterol, cholesterol, sitosterol, stigmasterol; alcohols namely octacosanol and triacontan-1-ol; and alkane hentriacontane. 7,8.

The present study undertakes the reinvestigations on the chemical examination of its leaves and we isolated one new ketone along with three known compounds, reported for the first time, from the methanol extract of the plant leaves.

The melting points were determined on Ganson Electrical Melting Point apparatus. 1H NMR spectra were recorded in CDCl₃ using tetramethylsilane (TMS) as internal standard on Bruker AC-300F 300 MHz NMR spectrometer and chemical shifts are given in δ (ppm). Pellets were prepared in KBr and IR spectra were recorded on Hitachi 570 infra red spectrophotometer. Mass spectra were recorded on VG-70S 11-250J GC-MS-DS Mass spectrometer.

Three kilograms dried leaves of *P. cineraria* were obtained from the Landscape Section HAU, Hisar and extracted with hot methanol. Extractives were subjected to column chromatography over silica gel using petroleum ether, benzene, ethyl acetate, methanol and their mixtures in the eluotropic series with increasing polarity. The silica gel (60-120 mesh) column chromatography of methanol extracts afforded four compounds, compound A to D (1, 2, 3 and 4, Fig. 1).

Compound A (methyl docosanoate, 1) was obtained from the eluate petroleum ether and it crystallized from methanol as a colourless solid, 11 mg, m. p. 55°, lit. m. p. 54°. IR ν max (KBr) (cm⁻¹): 476, 678, 735, 802, 865, 907, 909, 1261, 1404, 1646, 1717, 2361, 2854; 1H NMR (CDCl₃, δ) 3.75 (3H, s, –COOMe), 2.49 (2H, br s, –CH₂–COO–), 2.03 (2H, m, –CH₂–CH₂–COO–), 1.26 (36H, br s, 18×CH₂–), 0.89 (3H, m, 7.0 Hz, –CH₃); MS (m/z, relative intensity) 356 (M⁺, 1), 326 (1.5), 295 (1.5), 281 (3.8), 257 (11.3), 213 (18.9), 111 (41.5), 97 (74.5), 83 (100). A comparison of data of compound A fully agreed with the literature data of methyl docosanoate which is being reported for the first time from *P. cineraria* leaves.

Compound B (diisopropyl-10,11-dihydroxyicosane-1,20-dioate, 2) was obtained on elution with benzene-hexane (1:3) and it crystallized from methanol as a white crystalline solid, 20 mg, m. p. 88°; Found C, 68.10; H, 10.90. C₂₆H₅₀O₆ Required: C, 68.12; H, 10.91%; IR ν max (KBr) (cm⁻¹): 669, 722, 802, 1027, 1099, 1466, 1724, 2359, 2849, 2919, 3435; 1H NMR (CDCl₃, δ) 4.20 – 3.30 (4H, m, 2×COOCH Me₂, 2×CHOH–), 2.35 (4H, t, J 7.5 Hz, 2×CH₂–COO–), 1.60 (4H, br s, 2×CH₂–CH₂–COO–), 1.26 (24H, br s, 12×CH₂–), 0.88 (12H, d, J 7.5 Hz, 2×CH–(C₃H₃)); MS (m/z, relative intensity) 458 (M⁺, 37), 418 (4.5), 387 (7), 298 (4.5), 284 (7.5), 257 (20.5), 241 (7), 227 (9), 213 (16.5), 197 (9), 183 (24), 167 (16.5), 149 (50), 129 (38), 111 (51), 97 (70), 81 (100). The data suggested the compound B to be diisopropyl-10,11-dihydroxyicosane-1,20-dioate (2). A survey of the literature reveals that this compound has not been reported earlier.

Compound C (tricosan-1-ol, 3) was obtained on elution with benzene-hexane (1:1). It crystallized from methanol as white crystalline solid, 40 mg, m. p. 75°, lit. m. p. 73.5–74.5°. IR ν max (KBr) (cm⁻¹): 724, 1062, 1121, 1467, 2359, 2848, 2919, 3307; 1H NMR (CDCl₃, δ) 3.64 (2H, t, J 7.0 Hz, –CH₂–OH), 1.54 (2H, br s, –CH₂–CH₂–OH), 1.26 (40H, br s, 20×CH₂–), 0.86 (3H, t, J 7.0 Hz, –CH₃); MS (m/z, relative intensity) 341 (M⁺, 1), 290 (9), 279 (11), 256 (14), 213 (11), 178 (60), 161 (33), 149 (64), 111 (41), 97 (65), 81 (100). The data suggested
the compound C to be tricosan-1-ol (3). It may be mentioned that $M^+ (340)$ was not observed rather $M^+ + 1 (341)$ was observed. On comparison, the data of the compound C was found to agree fully with the literature data\(^\text{10}\) of tricosan-1-ol.

Compound D (7,24-tirucalladien-3-one, 4) was eluted with pure benzene. It crystallized from methanol as a white solid, 10 mg, m. p. 114°, lit. m. p. 115-116°. IR $\nu_{\text{max}}$ (KBr) (cm$^{-1}$): 668, 803, 961, 1058, 1167, 1260, 1374, 1461, 1704, 2357, 2855, 2925; $^1{\text{H NMR (CDCl}_3, \delta}$) 5.40–5.00 (2H, m, 2×>C=CH–), 3.54 (2H, m, –CH$_2$–CO–), 1.83 (3H, s, =C(CH$_3$)–CH$_3$), 1.57 (3H, s, =C(CH$_3$)–CH$_3$), 1.30 - 2.40 (20H, m, 8×>CH$_2$ and 2×>CH–), 1.01 (3H, s, –CH$_3$); MS (m/z, relative intensity) 424 (M +, 2.6), 414 (6), 396 (5.3), 381 (5), 329 (5), 303 (5), 279 (11.5), 264 (7), 256 (22), 239 (8), 213 (19), 148 (75), 97 (82), 83 (100). The data suggested the compound D to be 7, 24-tirucalladien-3-one (4). The data of the compound D was found in full agreement with the literature data\(^\text{11}\) of 7, 24-tirucalladien-3-one.

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HPTLC Standardization of Tinospora cordifolia Using Tinosporaside

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A simple and reproducible high performance thin layer chromatography method for the determination of tinosporaside in *Tinospora cordifolia* was developed and is described. This method involves separation of compounds by TLC on pre-coated silica gel 60F 254 plates with a solvent system of toluene: acetone: water (5:15:1) and scanned using densitometric scanner in UV reflectance photomode at 220 nm. The linearity was observed in the range of 0.5 to 8 mg. The tinosporaside content of 0.40% w/w was observed in test sample. The average percentage recovery value of 99.24±0.49 was obtained. The proposed method being precise and sensitive can be used for detection, monitoring and quantification of tinosporaside in *Tinospora cordifolia*.

**Key words:** *Tinospora cordifolia*, tinosporaside, HPTLC

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