In the present study *Taverniera cuneifolia* roots were subjected to petroleum ether extraction and two compounds were isolated from petroleum ether soluble portion of the plant. The plant is collected from wild resources and authenticated. The dried plant material is separated into its parts and roots were used for extraction. Preliminary phytochemical investigations showed presence of steroid and triterpenes. Two compounds were isolated and purified by column chromatography. These compounds were identified as Beta sitosterol, and Lupeol by spectroscopic methods. Amount of these two compounds in different parts viz. roots, stem and leaves is determined by HPTLC.

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Keywords: Steroids, HPTLC, Lupeol, Chromatography, Extraction.

1. Introduction:
Genus *Taverniera* contains twelve species which are widely distributed over tropical countries. It’s species *Taverniera cuneifolia* is a only species available in India and commonly present. These are much branched undershrubs, 30-60cm tall; branches terete, minutely appressed pubescent. Leaves 1-foliate; petiolules 3.5mm long; stipules scarious, triangular, united deciduous. Leaflets orbicular to obovate, 6-12mm across, cuneate at base, mucronulate, glaucous,. Flowers in axillary, lax, 2-6 flowered racemes longer than the leaves. Calyx 4mm long, finely pubescent; teeth triangular as long as the tube, acute, the two upper larger than the three lower. Corolla red, 10-12mm long, standard veined with dark purple veins, emarginated. Pods 1-2 jointed; joint ovoid, transversely rugose and echinate. Seeds reniform. Frequent along stream banks, in drying ponds etc. flowering and fruiting December to February. They are reported to contain glycyrrhizin and also used as substitute for Liquorice by local people.

2. Material and Methods:
2.1 Collection Authentication and Drying of Plant Material
The plant was collected from roadside in Majalgaon (Maharashtra), near patrud place and authenticated by Herbarium Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. It is then dried under shade and separated into parts, roots stem and leaves then size reduction is done in grinder to coarse powder. It is then subjected to extraction.

2.2 Extraction of Plant Material
Extraction of Roots was carried out by hot continuous soxhlet extraction by using petroleum ether (bp 60-80°C) as extracting solvent. The extract thus obtained are filtered and concentrated by solvent evaporation at room temperature. Preliminary phytochemical studies of extract showed presence of steroids and triterpenoids. This extract is redissolved in petroleum ether and TLC studies are performed.

2.3 Thin Layer Chromatography of Petroleum Ether Extract
TLC is carried out precoated silica gel G layer by Merck. Various solvent systems were tried to optimize solvent system which gives better resolution. Detection was carried out by Anisaldehyde sulphuric acid reagent. Petroleum ether: ethyl acetate (9:1) gave better resolution and its polarity can be increased by gradiently increasing portion of ethyl acetate viz. 7:3, 6:4 and so on in column chromatography.

2.4 Column Chromatography:
Column chromatography of above extract is carried out in glass column of internal diameter 34mm and stationary phase used is silica gel 230-330 mesh size (particle size-0.037-0.063 mm). Column is set in Petroleum Ether (60-
80): Ethyl acetate (9:1) and eluted. Gradient elution is followed. 35 fractions of 20 ml each were collected. A TLC study of these fractions was performed and fractions showing similar TLC patterns were mixed. The TLC pattern of initial 5 and 6 and showed single spot so they were mixed (Compound 1). TLC of fractions 15-17 showed same TLC pattern sp they were mixed and again subjected to column chromatography using small column of 12 mm diameter. The column was set with same solvent system and fractions of 1 ml each were collected. Fraction No. 19 and 20 were shown single spot at same Rf. This compound (Compound 2) was subjected to spectroscopic studies.

### 2.5 Spectroscopic studies of compounds:

UV spectroscopy is carried out at Y.B. Chavan College of Pharmacy, Aurangabad on Jasco UV spectrophotometer and IR spectroscopy is carried out at Y.B. Chavan College of Pharmacy, Aurangabad on Shimadzu (Japan) by KBr disc method. Mass and NMR spectroscopy is carried out at IIT, Powai.

#### 2.5.1 Compound 1

M.W.: 426, M.F.: C30H50 M.P: 214-216 °C, UV(Hexane): 350nm, IR ν: max cm⁻¹ (KBr): 3319, 2923, 2850, 1660, 1466, 1440 1198, 1042, 680, 750 3450 (OH), 3055, 1650 cm⁻¹ were observed. The IR spectrum absorptions for hydroxyl group (3450 cm⁻¹) and trisubstituted double bonds (3055, 1650 and 810 cm⁻¹) were observed. In the EI-MS spectrum of compound 2 molecular ion peak 414 corresponds to Mol. Wt. of the compound and characteristic fragment ions were observed at m/z 399, 396, 381, 329 and 303. The last two ions were diagnostic for sterols having Δ5-unsaturation. Other important fragments were observed at m/z 273 and 255, indicating the loss of [M-side chain] and [M-side chain-H2O], respectively. The 1H-NMR spectral data showed six methyl groups out of which two were tertiary (δ 0.68 and 1.64), three secondary (δ 0.93, 0.83 and 0.81) and a primary (δ 0.84). From above data Compound 1 is found to be Lupeol (Fig.1) and Compound 2 is Beta sitosterol (Fig.2).

#### 2.5.2 Compound 2

M.W.: 414.71, M. F.: C29H48O, Mp: 142°C, UV: λ max nm(Hexane): 208, IR: v max cm⁻¹ (KBr): 3450 (OH), 3055, 1650, 810 (C=C), EI-MS m/z : 414, 399, 396, 381, 329, 303, 275, 273, 255 1HNMR (CDCl3) (300MHz) (δ ppm): 5.13 (1H, m, H-11), 3.50 (1H, m, H-18), 2.28 (1H, dd, H-17), 2.21(1H, m, H-17), 1.95 (2H, d, H-10, H-6), 1.81 (1H, m, H-14, H-15), 1.64 (1H, m, H-28), 0.93 (1H, m, H-24), 0.92 (3H, d, H-21), 0.91 (1H, d, H-9), 0.84 (3H, t, Me-29), 0.83 3H, d, Me-26). 0.81 (3H, d, Me-27). 0.68 (3H, s, Me-18).

The compound was isolated as colorless needles. In the IR spectrum absorptions for hydroxyl group (3450 cm⁻¹) and trisubstituted double bonds (3055, 1650 and 810 cm⁻¹) were observed. In the EI-MS spectrum of compound 2 molecular ion peak 414 corresponds to Mol. Wt. of the compound and characteristic fragment ions were observed at m/z 399, 396, 381, 329 and 303. The last two ions were diagnostic for sterols having Δ5-unsaturation. Other important fragments were observed at m/z 273 and 255, indicating the loss of [M-side chain] and [M-side chain-H2O], respectively. The 1H-NMR spectral data showed six methyl groups out of which two were tertiary (δ 0.68 and 1.64), three secondary (δ 0.93, 0.83 and 0.81) and a primary (δ 0.84). From above data Compound 1 is found to be Lupeol (Fig.1) and Compound 2 is Beta sitosterol (Fig.2).

[Fig.1: Lupeol](image)

[Fig.2: Beta sitosterol](image)
solution 1mg/ml, out of this series of volumes is applied as tracks.

- **Preparation of test solution:** Test solution is prepared by dissolving 20mg of petroleum ether extract of *Taverniera cuneifolia* leaves stem and roots individually in 10ml of petroleum ether as solvent.

- **Stationary phase:** Precoated layer silica gel 60 F 254 from Merck of plate size 20.0 x 10.0 cm is used.

- **Mobile phase:** Petroleum ether: Ethyl acetate (9:1)

- **Development:** It is done in Twin Trough Chamber (20x10cm) made up of glass of volume 10.0 ml

2.6.2 Procedure:

Standard solution thus prepared was having concentration of 1mg/ml. This standard solution was applied on TLC plates at specific volumes say 8µl, 12µl, 16µl, 20µl and 24µl etc. which means 8µg, 12µg, 16µg, 20µg and 24 µg of standard respectively is applied on TLC plates at specific volumes say 8µl, 12µl, 16µl, 20µl and 24µl etc. This standard solution was applied on TLC plates. Along with these test solutions were applied at specific constant concentration 10µl. this TLC plate is then subjected to densitometric scan and data obtained is analyzed by regression. Standard Calibration graph is prepared and amount of these compounds in test sample is determined.

2.6.3 Estimation of percentage of Lupeol in *Taverniera cuneifolia*:

- **Parts used:** Leaves, Stem and Roots

- **Preparation of standard solution:** Standard solution of Lupeol is prepared by dissolving 10mg in 10 ml of solvent (Petroleum ether). Concentration of standard solution 1mg/ml, out of this series of volumes is applied as tracks.

- **Preparation of test solution:** Test solution is prepared by dissolving 20mg of petroleum ether extract of *Taverniera cuneifolia* leaves stem and roots individually in 10ml of petroleum ether as solvent.

- **Stationary phase:** Precoated layer silica gel 60 F 254 from Merck of plate size 20.0 x 10.0 cm is used.

- **Mobile phase:** Toluene: Ethyl acetate: Formic acid (9:1:0.1)

- **Development:** It is done in Twin Trough Chamber (20x10cm) made up of glass of volume 10.0 ml

Table 1: Calculation of amount of Lupeol in *T. cuneifolia* by regression

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rf</th>
<th>Amount</th>
<th>Area</th>
<th>X (Calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupeol</td>
<td>0.94</td>
<td>12.00 µg</td>
<td>193.47</td>
<td></td>
</tr>
<tr>
<td>Lupeol</td>
<td>0.94</td>
<td>16.00 µg</td>
<td>472.38</td>
<td></td>
</tr>
<tr>
<td>Lupeol</td>
<td>0.94</td>
<td>20.00 µg</td>
<td>671.53</td>
<td></td>
</tr>
<tr>
<td>Lupeol</td>
<td>0.94</td>
<td>24.00 µg</td>
<td>792.80</td>
<td></td>
</tr>
<tr>
<td>Lupeol</td>
<td>0.95</td>
<td>32.00 µg</td>
<td>1297.52</td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>0.96</td>
<td></td>
<td>1297.52</td>
<td>21.31 µg</td>
</tr>
<tr>
<td>Stem</td>
<td>0.94</td>
<td></td>
<td>513.03</td>
<td>17.55 µg</td>
</tr>
<tr>
<td>Roots</td>
<td>0.97</td>
<td></td>
<td>4457.87</td>
<td>60.80 µg</td>
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</tbody>
</table>

Substance: Lupeol @ 520 nm

Regression via area: Linear

\[ Y = 763.2 + 190.9 \times X \]

\[ r = 0.99892 \quad sdv = 1.70 \]

2.6.4 Procedure:

Standard solution thus prepared was having concentration of 1mg/ml. This standard solution was applied on TLC plates at specific volumes say 12µl, 16µl, 20µl, 24µl and 32µl etc. which means 12µg, 16µg, 20µg, 24µg and 32 µg of standard respectively is applied on TLC plates. Along

3. Result and Discussion

3.1 Estimation of amount of Lupeol:

Amount of Lupeol was calculated by regression as shown in Table 1. It showed linear regression as shown in Fig. 3

3.2 Calculation of amount of Beta sitosterol:

Amount of beta sitosterol was calculated by regression as shown in Table 2. It showed linear regression as shown in Fig. 4

Table 2: Calculation of amount of Beta sitosterol in *T. cuneifolia* by regression:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rf</th>
<th>Amount</th>
<th>Area</th>
<th>X (Calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α sitosterol</td>
<td>1.50</td>
<td>08.00 µg</td>
<td>2354.33</td>
<td></td>
</tr>
<tr>
<td>β sitosterol</td>
<td>1.50</td>
<td>12.00 µg</td>
<td>3011.51</td>
<td></td>
</tr>
<tr>
<td>α sitosterol</td>
<td>1.50</td>
<td>16.00 µg</td>
<td>3745.03</td>
<td></td>
</tr>
<tr>
<td>β sitosterol</td>
<td>1.50</td>
<td>20.00 µg</td>
<td>4594.42</td>
<td></td>
</tr>
<tr>
<td>β sitosterol</td>
<td>1.51</td>
<td>24.00 µg</td>
<td>5360.30</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount</th>
<th>Area</th>
<th>X (Calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves 1</td>
<td>1.48</td>
<td>105627</td>
<td>7.20 µg</td>
</tr>
<tr>
<td>Leaves 2</td>
<td>1.48</td>
<td>0751.68</td>
<td>7.20 µg</td>
</tr>
<tr>
<td>Stem 1</td>
<td>1.48</td>
<td>2519.92</td>
<td>9.20 µg</td>
</tr>
<tr>
<td>Stem 2</td>
<td>1.51</td>
<td>2431.20</td>
<td>8.73 µg</td>
</tr>
<tr>
<td>Roots 1</td>
<td>1.51</td>
<td>2665.47</td>
<td>9.96 µg</td>
</tr>
<tr>
<td>Roots 2</td>
<td>1.47</td>
<td>2840.51</td>
<td>10.88 µg</td>
</tr>
</tbody>
</table>

Standard: β sitosterol @ 520nm

Regression via area: Linear

\[ Y = 763.2 + 190.9 \times X \]

\[ r = 0.99892 \quad sdv = 1.70 \]

Fig. 3: Linear regression of Lupeol and of samples.

Fig. 4: Linear regression of standard and *T. cuneifolia* samples

From HPTLC data amount of Lupeol in leaves, stems and roots is found to be 2.13%, 1.75%, 6.08% respectively. Percentage of Beta sitosterol is found to be 0.72%, 0.89%
and 1.04% in leaves, stems and roots respectively. Lupeol possesses antioxidant, antitumor and antimalarial activities. Beta sitosterol is reported to have antiinflammatory, antioxidant, antitumor, hepatoprotective activities. These activities will be possessed by the plant.

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Source of support: Nil; Conflict of interest: None declared