Chapter 6

Isolation of β-sitosterol from leaves of *Basella rubra* Linn

6.1 Introduction

Beta-sitosterol is a phytosterols or plant sterol. The structure of beta-sitosterol is similar to that of cholesterol. Beta-sitosterol differs from cholesterol by the presence of an extra ethyl group.

There are many plant sources of beta-sitosterol, but the most important are wheat germ, rice bran, flax seeds, peanuts, soybeans, pumpkin seeds and corn oil. Muli and co-workers showed that a plant-based diet, rich in fibre, soy and flax seed, can increase serum levels of beta-sitosterol.

Beta-sitosterol is mainly known and used for its cholesterol lowering property. But studies have shown that the phytochemical may have other health benefits: easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity.

Beta-sitosterol is used for heart disease and high cholesterol. It is also used for boosting the immune system and for preventing colon cancer, as well as for gallstones, the common cold and flu (influenza), HIV/AIDS, rheumatoid arthritis, tuberculosis, psoriasis, allergies, cervical cancer, fibromyalgia, systemic lupus erythematosus (SLE), asthma, hair loss, bronchitis, migraineheadache, and chronic fatigue syndrome.
Some men use beta-sitosterol for enlarged prostate (benign prostatic hyperplasia or BPH). Some women use it for symptoms of menopause. It is also used for enhancing sexual activity. Marathon runners sometimes use beta-sitosterol to reduce pain and swelling after a run.

Beta-sitosterol is also used as a homeopathic remedy for certain male-associated problems that result from male hormones, including BPH and male pattern baldness. The mechanism for these positive effects appears to be the ability of this substance to lower circulating levels of certain male hormones. One drawback to the lower hormone levels, however, is that the side effects of large doses of this substance may include a lack of interest in sexual activity and may even contribute to the development of erectile dysfunction (ED). Some people apply beta-sitosterol to the skin for treating wounds and burns.

6.2 Extraction and Purification of the compounds obtained from the leaves of the plant

The leaves of *Basella rubra* Linn. were air dried and powdered. The powdered leaves (1.0 kg) were extracted by treating with acetone for 6-7 days, the colour of the solution changed to deep green. The solution was filtered, distilled to give a deep green semi-solid mass which was subjected to TLC using petroleum ether-ethyl acetate (8 : 2), showed five spots. The deep green semi-solid was subjected to silica gel column chromatography (16 cm Glass Column packed with 30g of 100-200 mesh Silica Gel) employing petroleum ether-ethyl acetate (8 : 2) as eluent. From the column, four compounds were isolated – an off white compound (7), a greenish white solid (8), a colourless solid (later identified to be Compound 6, discussed in previous chapter) and a light yellow compound (9).
The various fractions collected from the column chromatography of the acetone extract of the leaves of *Basella rubra* Linn., according to their decreasing order of polarity, are shown in the Table 6.1.

**Table 6.1:** The various fractions collected from the column chromatography of the acetone extract of the leaves of *Basella rubra* Linn

<table>
<thead>
<tr>
<th>Eluent</th>
<th>Fraction No.</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>Petroleum ether (100%)</td>
<td>1-10</td>
<td>Viscous solid</td>
</tr>
<tr>
<td>Petroleum ether (100%)</td>
<td>11-16</td>
<td>Off white solid, 7</td>
</tr>
<tr>
<td>Pet.ether:chloroform (95:5)</td>
<td>17-37</td>
<td>Off white solid, 7</td>
</tr>
<tr>
<td>Pet.ether:chloroform (90:10)</td>
<td>38-55</td>
<td>Off white solid, 7</td>
</tr>
<tr>
<td>Pet.ether:chloroform (90:10)</td>
<td>56-67</td>
<td>Greenish solid, 8</td>
</tr>
<tr>
<td>Pet.ether:chloroform (90:10)</td>
<td>68-98</td>
<td>Colourless solid, 6</td>
</tr>
<tr>
<td>Pet.ether:chloroform (87:13)</td>
<td>99-113</td>
<td>Light yellow solid, 9</td>
</tr>
</tbody>
</table>

On elution of the column of the acetone extract of the leaves of *Basella rubra* with petroleum ether-ethyl acetate (95 : 5), a semisolid grayish compound 7, which was recrystallized from methanol to give a colourless compound, was isolated and it was found to be β-sitosterol. The structure of β-sitosterol, 7 was confirmed by comparing with the spectral data of the authentic sample.
β-sitosterol

Solid Compound.

IR (KBr): \( \nu_{\text{max}} \) 3413, 2926, 1642, 1464, 1379, 1260 and 1168 cm\(^{-1}\);

\(^1\)H NMR (CDCl\(_3\) : CD\(_3\)OD = 4:1): \( \delta \)H 0.82 (H - 29), 0.85 (m, H - 26 & 30), 0.85 (m, H - 20, H - 27), 0.87 (s, H - 9), 0.91 (br., H - 24), 1.92 (m, H - 3, H - 14, H - 15), 1.80 (m, H - 2, 3, 11), 2.00 (m, H - 2, H - 10), 2.25 (m, H - 3, H - 17), 1-1.5 (overlapping peaks due to rest of the protons), 3.58 (m, H - 1), 5.37 (br s, H - 12);

\(^{13}\)C NMR (CDCl\(_3\) : CH\(_3\)OD = 4:1): \( \delta \)C 71.8 (C - 1), 29.1 (C - 2), 36.1 (C - 3), 39.7 (C - 4), 51.2 (C - 5), 37.2 (C - 6), 56.0 (C - 7), 42.3 (C - 8), 55.9 (C - 9), 28.2 (C -
10), 26.0 (C − 11), 121.7 (C − 12), 140.7 (C − 13), 31.9 (C - 14), 33.9 (C – 15), 24.3
(C − 16), 36.4 (C − 17), 24.3 (C − 19), 24.6 (C − 20), 31.8 (C − 21), 31.6 (C − 22),
20.0 (C -23), 45.8 (C -24), 29.1 (C − 25), 18.8 (C − 26), 18.7 (C − 27), 21.0 (C-28),
12.5 (C-29), 19.1(C-30);

The spectra of the compound β - sitosterol are presented in the Appendix

6.4 Conclusion

The isolation and characterisation of β - sitosterol is reported for the plant

*Boswellia rubra*