Chapter -6

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Transverse section of fruit shows the presence of mesocarp, endocarp and endosperm. Seed shows the presence of endocarp, sclereids, and endosperm and starch grains. The powder microscopy of fruit and seed show lignified sclereids, fragment of endosperm, starch grains, fibres, crystals and xylem vessels. Histochemical tests of fruit reveal the presence of lignins, starch and phenols which are characterised by different histological zones. Physico-chemical studies such as ash value, extractive value, moisture content, powder behaviour and fluorescence analysis were carried out on both fruit and seed. Total ash, water soluble and methanol soluble extractive values were found more in fruit than seed. Such information from the pharmacognostic study can act as reference information for correct identification of particular plant and useful in making a monograph of the plant.

There was significant wound contraction by methanolic extract of Barringtonia acutangula (MEBA) fruit. It increases the number of fibroblasts, collagen tissue and causes complete epithelialization. The percentage of wound contraction significantly decreases when the extract were applied on infected wound. Diabetic rats show delayed epithelialization period compared to non-diabetic rats. In our study, MEBA significantly decreased the epithelialization period. The histopathological study reveals that MEBA 20% w/w ointment increases number of fibroblasts, collagen tissue and causes complete epithelialization. So, MEBA may have significant effect on the proliferative phase of wound healing in diabetic rats. An increase in tensile strength and hydroxyproline content of treated wounds in the present study may be due to increase in collagen concentration and stabilization of fibres.

The GC-MS chromatogram confirmed the presence of 10 compounds with the retention time of 8.673, 13.307, 36.138, 37.889, 41.161, 41.377, 42.236, 42.959, 43.166 and 43.665. Out of the ten compound identified, the most prevailing compounds were glycerin (32.25%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(14.22%), 9,12-Octadecadienoic acid (Z,Z)-( 15.63%), 6-Octadecenoic acid, (Z)-(14%) and n-Hexadecanoic acid (12.52%). The rest five minor compounds were Octadecanoic acid
(3.33%), 8-Octadecenoic acid, methyl ester (3.11%), hexadecanoic acid, methyl ester (2.44%), 8, 11-Octadecadienoic acid, methyl ester (1.83%) and Octadecanoic acid, methyl ester (0.66%).

The methanolic fruit extract of *Barringtonia acutangula* showed antibacterial activity against gram positive and gram negative bacteria. So it may possess broad spectrum activity. The antimicrobial activity of methanol extract of *Barringtonia acutangula* fruit may be attributed due to presence of bioactive compounds such as Glycerin, 9,12-Octadecadienoic acid (Z,Z)-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, n-hexadecenoic acid, Octadecanoic acid, 8-octadecenoic acid methyl ester, Octadecanoic acid, methyl ester and Hexadecanoic acid, methyl ester, which are in agreement with the previous report.

Binding mode analysis showed the compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (CAS-28564-83-2, docking score: -6.61) had showed very good binding affinity in comparison to hydroxyproline (docking score: -4.6) and other constituents of *Barringtonia acutangula* fruit extract. The better activity of compound CAS-28564-83-2 might be due to an extra hydroxyl group present on the compound and also the presence of a double bond in the ring make the compound more rigid and it get better fit into the active site pocket where as hydroxyproline is more flexible. The inactiveness of the other compounds may be due to the lack of hydrogen bond interactions in active site pocket with Leu188.

Thus, the present study justifies the potent wound healing activity of the phytoconstituents present in *Barringtonia acutangula*, thereby justifying its traditional use. The wound healing activity of methanolic extract of *Barringtonia acutangula* in normal, infected and diabetic rats may be attributed to the presence of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and n-hexadecenoic acid for their GSK3-β inhibition activity and antibacterial activity.