ABSTRACT

*Barringtonia acutangula* belongs to family Barringtoniaceae found throughout India. Fruits are bluntly quadrangular, long and broadest in the middle, broader angle and rounded. Traditionally, fruits of *Barringtonia acutangula* are used in diseases of the blood, bronchitis, sore eyes, headache, hallucinations, abdominal colic, syphilis, nasal catarrh, wound, ulcer, leprosy, cough, dysmenorrhea, etc. There are reports of antidiabetic, antioxidant, anti-inflammatory, cytotoxicity, anti-arthritis, antimicrobial, antibacterial activity of *Barringtonia acutangula*. The fruits are used as wound healing agents by local people of western Odisha. However, thorough literature survey reveals that there is no scientific report on wound healing activity of *Barringtonia acutangula*.

The present study aims at pharmacodynamic evaluation of wound healing potential of methanolic extract of *Barringtonia acutangula* to validate its folklore claim. Wound healing activity was evaluated by using excision and incision wound model in normal rats with or without infection and experimentally (streptozotocin) induced diabetic rats. The parameters used to access wound healing activity in this study are: Percentage wound closure of excised wound, tensile strength of incised wound, histopathology of excised wound, and hydroxyproline estimation of excised wound. This was followed by antibacterial activity study, GC-MS study and molecular docking study. A detailed pharmacognostic study of the fruits was done to establish their correct identity and to prevent any adulteration.

The Gram positive bacterial strains of *E. faecalis*, *S. aureus*, *A. bumannii* and Gram negative bacterial strains *C. freundii*, *E. Aerogenes*, *E. Coli* were used to assess antibacterial activity. Nutrient agar and broth were used for this study. Zone of inhibition was calculated by disc diffusion method and minimum inhibitory concentration and minimum bactericidal concentration were calculated by tube dilution method.

Wingless type (Wnt) signaling pathway or β-catenin pathway can enhance wound healing through the inhibition of glycogen synthase kinase 3-β (GSK3-β), an important regulatory protein. GC-MS analysis of active plant extract was done to know the bioactive
components present in it. This was followed by molecular docking of the constituents with GSK3-β to identify the compounds responsible for wound healing.

Transverse section of fruit shows the presence of mesocarp, endocarp and endosperm. Seed shows the presence of endocarp, sclereids, 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. 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. In histopathological studies, a greater degree of epithelialization, collagen and fibroblastic deposition was observed in methanolic extract treated rats. In the control group wounded area skin shows ulceration with inflammation exudates. There is mild increase in fibrous tissue as well as granulation tissue and congested blood vessels. Wound healing is incomplete. In 20%w/w methanolic extract treated rats intact portion of skin reveals normal histology. Since methanolic extract increases the number of fibroblasts, collagen tissue and causes complete epithelialization, they may have a significant effect on the proliferative phase of wound healing. The percentage of wound contraction significantly decreases when the extract were applied on infected wound. The presence of systemic infection may have decreased the immunity because of which there was such a delay in wound healing.

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.

Methanolic extract of *Barringtonia acutangula* (MEBA) significantly increased the hydroxyproline content thereby influencing the collagen turnover. In addition methanolic extract of *Barringtonia acutangula* (MEBA) fruits increase tensile strength in dose dependent manner. This shows its effectiveness in wound healing of diabetic rats. There
are reports of positive effects of combination of transforming growth factor and fibroblast growth factor on biochemical parameters of wound healing and the tensile strength deficit of diabetic wounds. 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. Hydroxyproline strongly inhibited GSK-3β. Hydroxyproline, is a specific marker of collagen and an important component of the extracellular granulation tissue matrix influencing rapid collagen turnover and accumulation. Hydroxyproline also helps in promoting the cutaneous wound healing through the elicitation of β- catenin-dependant Wnt pathway through the inhibition of GSK3-β, explaining the increased rate of wound contraction. As the structure of 4H-Pyran-
4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- is very much similar with hydroxyproline there is anticipation that compound may likely to play a major role in healing activity.

Thus, the present study justifies the potent wound healing activity of the phytococonstituents 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.