Chapter 1

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Plant species with medicinal value are known to be used for treatment and cure of different diseases since time immemorial (Willcox et al. 2004). Though we have number of synthetic drugs and antibiotics, 80% of the world population still rely on medicinal plants (Lambert et al. 1997; Goud et al. 2005; Khan et al. 2009). The high cost, the toxicity and adverse effect associated with synthetic drugs and antibiotics forced us to show interest in medicinal plants (Willcox et al. 2004). Medicinal plants are used in three ways. Some of the modern medicine like morphine, atropine, quinine, artemisinin, colchicine, digoxin, ephedrine, reserpine, vincristin and taxol etc are derived from plant (Ramawat et al. 2008). Some traditional medicines with proven efficacy are available in written treatise text such as Ayurveda, Unani, Siddha system of medicine (Dahanukar et al. 2000). In addition, there are some folklore claims based on traditions practiced by tribal people, folk healers, vaidyas and local villagers. Many of these folklore claims are not scientifically justified yet (Dahanukar et al. 2000).

India is one of the mega biodiversity countries of the world. It has very rich flora and fauna. There are about 17,500 species of flowering plants, 140 genera and 5285 species are native to the country. Many of these plants are used traditionally for treatment or cure of human and animal ailments (Dahanukar et al. 2000). Most herbal drugs use leaves (34%) followed by bark (15%), entire plant (12% mostly in case of smaller herbs), underground plant parts (10% including tuberous, rhizome and bulb) and exudates like latex, gum or resin (8%) (Ramawat et al. 2008).

Lack of standard of efficacy and safety in traditional medicinal plants or herbal drugs is an issue. They are inconsistent in quality. They show various pharmacological properties and phytochemical profile. Their active principles are not known (Ramawat et al. 2008). There are limited studies on traditional drugs with modern standards of safety and efficacy (Salim et al. 2008).
There are two approaches to develop successful drugs from medicinal plants: phytotherapeutic approach and the phytochemical approach. In the phytotherapeutic approach, the extraction, fractionation, isolation and separation of chemical entities from plant materials are guided by pharmacological activities. This approach is used to discover new compounds as medicine as it helps in the development of crude plant materials as standardised drugs (Mendonca-Filho et al 2006).

In the phytochemical approach, the plant materials are subjected to chemical analysis and individual chemical compounds are isolated and characterized. Then their pharmacological activities are evaluated and are practiced based on their chemical structure (Ramawat et al 2008). Phytochemicals are chemicals extracted from plants. They may be primary or secondary metabolites. Primary constituents include the common sugars, proteins, aminoacids, purines and pyrimidines of nucleic acids, chlorophylls etc, that are essential for growth of metabolites of plants. Secondary metabolites are tannins, saponins, alkaloids, flavonoids, anthraquinones, cardiac glycosides, cyanogenic glycosides etc (Ramawat et al 2008).

There are standardised methods to evaluate pharmacological parameter. Initially in vivo bioassay is done using animal experimental models simulating human disease to determine efficacy and safety of herbal drugs. This is followed by in-vitro assay to reflect in vivo effect using isolated tissue or organ (Salim et al 2008, Mendonca-Filho et al 2006; Yadav et al 2008).

Wound is a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection and is produced due to any accident or cut with sharp edged things. It may occur due to thermal, chemical, physical, microbial or immunological exploitation of the tissues (Sabale et al 2012). If it is not treated immediately, it can lead to microbial infections (Pandian et al 2013). The wound infections are most common in developing countries due to poor hygienic condition. The microorganisms like gram positive and gram negative bacteria are the principal pathogen of wound infections. Diabetic patients are at increased risk of developing infection (Edan et al 2010). This is due to impaired leukocyte function associated vascular diseases, poor glucose control and altered immune response (McMahon et al 1995, Bhatia et al 2003).
Wound healing is a complex phenomenon for the regain or restoration of disrupted anatomical continuity and disturbed functional status of the skin (Begum et al 2000), accomplished by several processes which involve different phases including inflammation, granulation, fibro genesis, neo-vascularization, wound contraction and epithelisation (Clark et al 1996). The basic principle of optimal wound healing is to minimize tissue damage, provide adequate tissue perfusion, oxygenation, proper nutrition, moist wound healing environment (Pierce et al 1995). The main aim of wound therapy is to enhance wound healing in the shortest time possible, with minimal pain, discomfort and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration (Blower et al 2001).

Gandhamardan Hills or Gandhamardan Parbat is located in between Balangir and Bargarh district of western Odisha. This hill is well known for medicinal plants. The Gandhamardan mountain ranges are a rich source of diversity for medicinal plants. The Botanical Survey of India has reported the existence of 220 plant species of medicinal value. Local people, however, claim that there are more than 500 species of medicinal plants in this area (Wikipedia). *Barringtonia acutangula* (Local name- Hinjolo) is one of those medicinal plants available in Gandhamardan Hills.

*Barringtonia acutangula* belongs to family Barringtoniaceae found throughout India. Fruits are bluntly quadrangular, long and broadest in the middle, broader angle and rounded (Anonymous 1988). 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, dysmenorrhoea etc (Joshi 2003) There are reports of antidiabetic, antioxidant, anti-inflammatory, cytotoxicity, anti-arthritis, antimicrobial, antibacterial activity of *Barringtonia acutangula*. (Khatib and Patil 2011; Muralidhar et al 2013; Jalaluddin and Banik 2014).

The fruits are used as wound healing agents by local people of western Odisha. They crushed the matured fruits to make paste and applied the paste topically on the surface of the wound. However, thorough literature survey reveals that there is no scientific report on wound healing activity of *Barringtonia acutangula*. So the present study is an attempt to evaluate wound healing activity of different extracts of its fruit.
Petroleum ether extract of the stem bark (Rahman et al 2005), ethanolic extracts of seed (Sahoo et al 2008), ethanolic extract of leaf (Padmavathi et al 2012) of *Barringtonia acutangula* showed potential antibacterial activity and n-hexane extract of the leaf possess potential antifungal activity (Bharati et al 2010). However, there are no reports of antimicrobial activity of its fruits. When a wound occurs and is exposed to external environment, it is more prone to be attacked by microbes, which invade through the skin and delay the natural wound healing process (Ready et al 2008). So in addition to wound healing activity, is also evaluated the antibacterial activity of the most potent extract.

Aqueous ethanolic extract of roots (Nilesh et al 2010), ethanol and aqueous extracts of leaf (Marslin et al 2014), and aqueous extract of fruit significantly decreases blood glucose levels in Streptozotocin (STZ) induced hyperglycemic rats (Khatib and Patil 2011). Ethanol leaf extract significantly decreased 40-50% of the elevated blood glucose level, cholesterol, triglycerides, urea, creatinine, and bilirubin in alloxan (150mg/kg) induced diabetic rats (Palanivel et al 2013). The development of wounds is a serious complication for patients with diabetes (Lavery et al 2007). Hyperglycemia can increase infection and impair wound healing (O Dell, 1999). So it is planned to evaluate the wound healing activity of fruits in experimentally induced diabetic rats.

In-silico docking studies play an important role in the identification of lead molecules in drug discovery. There are several studies showing that Wingless type (Wnt) signaling pathway or β-catenin pathway can enhance wound healing (Naika et al 2015; Rao et al 2015) 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.

So the present study is undertaken with following objectives.

- To evaluate the wound healing activity of different fruit extracts of *Barringtonia acutangula* in normal albino rats with or without infection.
- To evaluate the wound healing activity of different fruit extracts of *Barringtonia acutangula* in experimentally induced diabetic albino rats.
➢ To evaluate the pharmacognostical (transverse section & powder microscopy) study of *Barringtonia acutangula* fruit.

➢ To study antibacterial activity of potent fruit extract of *Barringtonia acutangula*.

➢ To study the GC-MS of potent fruit extract of *Barringtonia acutangula*.

➢ To study the molecular docking of the active constituents of fruit of *Barringtonia acutangula*.

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 assess 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. Antibacterial activity of the potent extract was evaluated using both gram positive and gram negative bacteria. Six bacterial strains namely *E. Faecalis, S aureus, A. baumannii, C. Freundii, E. Aerogene* and *E coli* were used to study antimicrobial activity. GC-MS analysis of active plant extract was done followed by molecular docking of the constituents to know the mechanism of action. Before that a detailed pharmacognostic study of the fruits was done to establish their correct identity and to prevent any adulteration.