12.1. Introduction

Burn wound treatment is one of the challenges in the medical science. In burn area, excessive inflammation leads to increase vascular permeability, local tissue edema and neutrophil activation that cause local tissue damage (Ames et al., 1993). New wound healing compounds are welcomed for accelerating regenerating process for its anti-inflammatory and antimicrobial effects. The interest in natural antioxidants has been increased considerably. As resources of natural antioxidants much attention has been paid to plants (Dean and Davies, 1993). The antioxidant activity of several Iranian plants has been reported (Tutour, 1990).

Proper and timely wound healing is a vexing problem faced by all clinicians. In majority of patients normal healing establishes tissue integrity quickly and effectively. Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical processes, which occur in living organism. Repair through regeneration is very common in unicellular and the lower metazoan animal groups while it is highly restricted in the higher animals (Sharief and Rao, 2007).

The treatment of wound healing became human’s interest since a long time ago. From east to the west, it became a universal subject when human started to use various kinds of medicinal plants as a wound healing treatment. For example, a bread mold is used in Chinese folk medicine to treat small burns which could be found in written records at least for 2000 years (Brown, 1972). Ibn Sina explained that papyrus is beneficial for haemorrhage to cease and fresh wounds to scar over. This practice is parallel to a hadith narrated by Abu Hazim who had heard Sahl b. Sa’d asked about
the treatment for Prophet Muhammad (PBUH)’s wound on the day of Uhud. Fatimah, his daughter burnt a piece of matting to ashes and applied it to the wound. The matting is made from papyrus that contains a strong caustic property that useful to heal wounds (Ibn Qayyim 2001).

When we trace back the Greek physician practice during Hippocrates (460-370 B. C. E.), the concept of combating and curbing bacterial infection in wound is still a major concern for wound healing treatment until nowadays. Previously, the physicians applied wine into the wound and later it was discovered that polyphenols, malvoside in wine was the compound responsible for bactericidal activity (Eaglstein et al., 2005). This practice is similar with the use of Calendula succus containing fresh juice of *Calendula officinalis* after surgery for enhancing wound healing process. This preparation is included in The Complete German Commission E Monographs for wound healing treatment (MacKay and Miller, 2003). Hendy *et al.* (2013) studied the wound healing properties of *Acrostichum aureum* and *Aspeciosumin* ethanolic extract. The result confirmed that topical application of both extracts shown significant effect in wound healing process.

Marco *et al.* (2016) isolated flavonoid oligoglycosides from *Ophioglossum vulgatum* which possesses wound healing properties. Two new glycosylated and acylated flavonoids, viz. quercitin 3- O-[(6 – caffeoyl)- B glucopyranosyl (1- 3) x – rhamnopyranoside] – 7 – O-X- rhamnopyranoside, and kaemferol-3-O-[(6-caffeoyl)- B- glucopyranosyl(1 -) x – rhamnopyranoside] – 7- O-X- rhamnopyranoside together with known quercitin – 3- O- methyl ether were isolated from the aerial parts of the fern *Ophioglossum vulgatum*. These 3 compounds were all found to be active in scratch wound healing assays on keratinocytes
How et al. (2011) examined wound healing activity in the aqueous extract of *Blechum orientale* on Sprague Dawley rats. The result confirmed that 25 (w/w) concentration was capable of producing significant wound healing activity. Ranjan et al. (2014) studied wound healing activity of *Drynaria quercifolia* rhizome. The rhizome was subjected to successive extraction using n-hexane, petroleum ether, chloroform and methanol and finally with distilled water using soxhlet extractor. They reported that the rate of wound contraction was significantly higher in the animals treated with methanol herbal extracts compared to the reference drugs (ie) Neosporin. Furthermore the methanol herbal (DQ) extract exhibited significantly decreased period of epithelisation compared to controls. Wound dressed with both methanolic extract and chloroform found to be epithelised fast.

Azam et al. (2015) studied the effect of *Equisetum arvense* ointment on wound healing activity. According to their results 35 *Equisetum arvense* ointment promoted wound healing and relieved pain during the 10 day period after episitomy. Manjunatha et al. (2007) reported the wound healing activity of aqueous and ethanol leaf extracts of *Lycopodium serratum*. The result confirmed that ethanolic extract treated animals showed faster epithelisation of wound.

Negahdari et al. (2017) examined the wound healing activity of extracts and formulations of *Aloe vera*, Henna, *Adiantum capillus-veneris*, and Myrrh on Mouse Dermal Fibroblast Cells - The present study demonstrated that the mentioned herbal extracts might be effective in wound healing, through the improvement in the migration of fibroblast cells and regulating the gene expression of *Tgfβ1* and *Vegf-A* genes in fibroblast cells treated with extracts. Lai et al. (2011) observed potential dermal wound healing agent in *Blechnum orientale*. The ethnotherapeutic use of this
The water extract of *B. orientale* is a potential candidate for the treatment of dermal wounds. Synergistic effects of both strong antioxidant and antibacterial activities in the extract are deduced to have accelerated the wound repair at the proliferative phase of the healing process.

Two species from the genus *Davallia*, *Davallia mariesii* and *Davallia solida* have been used as painkiller, anti-inflammatory agents and also for healing of fractured bones (Chang *et al*., 2007; Whistler, 1992a). *Dynaria fortunei* and *Microsorum scolopendria* belong to the same family have been used for skin inflammation, lumbago treatment and wound healing (Lee *et al*., 2008; Bloomfield, 2002). *Acrostichum aureum* is used to treat wound and ulcer (Morrison *et al*., 1994) while *Blechnum orientale* are being used as tonic and to cure wound cicatrization (Defilipps *et al*., 1988). The rhizome extract of *Drynaria quercifolia* was found to inhibit the growth of at least 6 bacteria strains significantly (Kandhasamy, 2008). Phytochemical studies revealed that Pteridophytes are rich in triterpenoids, sesquiterpenes, catechins, flavonoids, steroids and glycosides which are important source of therapeutic drugs (Anuja *et al*., 2010; Liu *et al*., 1999; Economides and Adam, 1998).

Most of the reported phytochemicals are known for their radical scavenging activity. *P. leucotomus*, has also been used to develop another skin product called “Fernblock” which is used as an anti-aging and sun-block cream. Several major compounds such as 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillin acid, caffeic acid, 4-hydroxy cinnamic acid, ferulic acid and five chlorogenic acid isomers had been identified from the *P. leucotomus* extract and they are used to standardize the product (Garcia *et al*., 2006). The success of these products indicates the potential use
of some ferns to be developed into pharmaceutical products to treat skin infections. With this knowledge the present study was aimed to carry out wound healing activity in chloroform extract of *A. latifolium*, *A. evecta* and *M. fraxinea*.

### 12.2. Materials and methods

#### 12.2.1. Preparation of plant extracts

Fresh and healthy plant parts of *Adiantum latifolium* Lam., *Angiopteris evecta* (Forst) Hoff. and *Marattia fraxinea* Sm. were collected, washed thoroughly under running tap water and then rinsed with distilled water. The plant materials were shade dried for 15 days at room temperature. The dried plant samples were crushed into fine powder and stored in airtight bottles. The powdered samples were extracted with various solvents (petroleum ether, chloroform, acetone and ethanol) using soxhlet apparatus with 1:6 ratio (w/v). 30 g: 180 ml solvents crude extracts were collected in petriplates and the solvents were evaporated to dryness, the residue left over was transferred to a small vial and stored at 4°C in refrigerator for further analysis.

#### 12.2.2. Grouping of Animals

For excision wound model, animals were divided into IX groups consisting of six animals in each group as follows: Group I – Simple ointment base; Group II – Animals treated with standard (povidine iodine ointment), Group III to VIII – test groups with 5% and 10% doses of *Adiantum latifolium*, *Angiopteris evecta* and *Marattia fraxinea* stem chloroform extracts.

#### 12.2.3. Wound healing study

Screening for wound healing activity was performed by excision wound model model without infection. All the test sample and standard drug were applied topically.
12.2.4. Excision wound model

Each group was anesthetized by open mask method with mild anesthetic ether. The rats were depilated on the back and a predetermined area of $500\text{mm}^2$ full thickness skin was excised in the dorsal inter scapular region. The areas of the wounds were measured (sq mm) immediately placing a transparent polythene graph paper over the wound and then tracing the area of wound on it taken as initial wound reading. All the test samples were applied once daily. The wound area of each animal was measured on days 0, 2, 4, 6, 8, 10, 12 and 14 after inflicting the wound. Wound contraction (WC) was calculated as a percentage change in the initial wound size (Morton, 1972).

The % of wound contraction (WC) = [(Initial wound size-specific day wound size)/Initial wound size] X 100.

12.3. Results

12.3.1. Excision wound model

Wound healing activity of *A. latifolium*, *A. evecta* and *M. fraxinea* were studied and recorded in Fig.12.1. The wound size was highly reduced in rats treated with standard when compared to that of control. The chloroform extract of *A. latifolium*, *A. evecta* and *M. fraxinea* were used to study the wound healing activity. The results showed decreased wound size in chloroform leaves extract of *M. fraxinea* and *A. latifolium* at 10% treatment. The wound size of treated rats gradually decreases both in control, standard and in the studied plant extracts. In present study, 10% of plant extracts of *A. latifolium* and *M. fraxinea* showed better results when compared to the control. Among the three plants, *M. fraxinea* showed significant wound healing activity.
Plate 12.4
Wound healing activity (Excision wound model) 1st day

Simple ointment treated

Povidone ointment treated

2.5% Ointment treated

2.10% Ointment treated

10.5% Ointment treated

10.10% Ointment treated

18.5% Ointment treated

18.10% Ointment treated
Plate 12.2
Wound healing activity (Excision wound model) 20th day

Simple ointment treated

Povidone ointment treated

2.5% Ointment treated

2.10% Ointment treated

10.5% Ointment treated

10.10% Ointment treated

18.5% Ointment treated

18.10% Ointment treated

The wound healing activity of *Marattia fraxinea* stem chloroform extracts showed the marked reduction in the area of the wound from 522.45 mm$^2$ to 91.27 mm$^2$ at 400 mg/kg concentration, next to that chloroform extracts of *Adiantum latifolium* showed reduced wound area from 523.32 mm$^2$ to 174.35 mm$^2$. The percentage of wound healing potential of stem chloroform extract of studied ferns are as follows: *M. fraxinea* (82%) > *A. latifolium* (67%) > *A. evecta* (37%).

### 12.4. Discussion

The effectiveness of the plant extract to enhance wound healing process might be associated with antibacterial activity of the plant (Lai *et al.*, 2009). And the mechanism of antiseptic in the wound treatment is suggested by minimizing bacterial infection that can faster wound healing process. Plants contain tannins, saponin, flavonoids and alkaloids shown the antimicrobial activity (Bandaranyake, 2002). Thus, tannins, saponins and flavonoids in these plants might responsible in the enhancing wound healing process. Flavonoids can reduce lipid peroxidation by improving vascularity and slowing the onset of cell necrosis (Getie *et al.*, 2002). The flavonoids in *A. speciosum* and *A. aureum* could enhance the wound healing process.
by increasing the strength of collagen fibers, circulation, preventing the cell damage, and promoting the DNA synthesis (Getie et al., 2002). In addition, tannins also play an essential role in wound healing process, where tannins will precipitate the proteinaceous matter and act as astringents drawing tissues and contracting them. These properties are used in treating inflamed mucous membranes characteristics of coughs, colds, alleviating intestinal infections and bathing wounds. As a topical application, tannins can be used to stop bleeding, reduce inflammation and heal the wounds (Ong, 2004). Polyphenolic flavonoids and tannins are reported to facilitate wound healing (Prabu et al., 2008). The phytochemical studies confirmed the existence of flavonoids and tannins in A. latifolium, A. evecta and M. fraxinea. The wound healing properties of A. latifolium, A. evecta and M. fraxinea is proportional to the existence of flavonoids and tannins.

12.5. Conclusion

In conclusion, this study has shown that chloroform extract of M. fraxinea stem possessed wound healing properties. Wounds treated with 10% M. fraxinea (T4) exerted faster wound contraction similar to the standard. Therefore, further studies with purified constituents are needed to understand the complete mechanism of wound healing activities in order to develop a novel plant-based ointment for wound healing treatment.