Wounds result in functional disruption of living tissues. Herbs and their extracts have potential to regenerate damaged skin. Purpose of this study was to evaluate the wound healing activity of aloe vera leaf extract in combination with curcuma longa extract. Both herbs possess anti-inflammatory and antibacterial properties. Gel-I and Gel-II were formulated using Carbopol 940, 3%w/w aloe vera leaf extract, 1%w/w and 3%w/w curcuma longa extracts respectively and were applied on excision and incision wounds created on albino rabbits. Animals were divided into four groups having six animals, group-I was control, group-II received standard gel (Nitrofurazone), group-III received Gel-I, group-IV received Gel-II. Gels were evaluated for color, pH, clarity, viscosity, consistency, spreadability, extrudability, skin irritation test and stability studies. Gels were applied on excision wound once a day for 12 days. Wound treated with Gel-II showed better healing activity and greater percentage of wound contraction (p<0.05) than 1%w/w. Histology was carried out on skin of 5th and 12th day incision wounds. Gel-II showed better epithelialization time, wound contraction, tensile strength. Wound healing activity was because of antibacterial, anti-inflammatory and antioxidant properties of phytoconstituents of aloe vera and curcuma longa.

**Keywords**: wound healing, aloe vera, curcuma longa, excision wound. incision wound.
I. INTRODUCTION
Poor hygienic conditions result in wound infection in many developing countries. (Kumar et al., 2006). Breaking and opening and of skin is caused by wounds which are actually physical injuries. Adequate method for wound healing is important for the maintenance of anatomical continuity and functional condition of the skin (Singh et al., 2006). Different steps involved in wound healing include appearance of coagulation, formation of granulation tissue, inflammation of wound, formation of matrix, remodeling of connective tissue, collagen formation and achievement of wound strength (Reddy et al., 2002). Across the world many traditional practitioners particularly in India and China have helpful information of many unknown and lesser-known wild plants for the treatment of burns and wounds (Kumar et al., 2007). In Africa and Asia, traditional forms of medicine being practiced for centuries and are scientifically investigated for their potential in the treatment of wounds related disorders (Krishnan, 2006). Traditional practitioners for herbal medicines have described therapeutic efficacy of different indigenous plants. (Natarajan et al., 2003). Natural products are a source of synthetic and traditional herbal medicine (Singh and Singh, 2001). According to Mukherjee and Biswas (Biswas and Mukherjee, 2003), About 70% of the Ayurvedic drugs used for wound healing are obtained from plant origin, 20% are from mineral origin, and 10% belong to animal products. Since the dawn of civilization, medicinal plants found to be the essential part of human life. Pharmacological studies have documented that the medicinal plants are potential source of bioactive compounds (Prusti et al., 2008). All novel drugs are obtained from natural medicinal sources that form the ingredients in traditional systems of medicine, food supplements, modern medicines, nutraceuticals and folk medicines (Ncube et al., 2008).

*Aloe Barbadensis* or *aloe vera* (Liliaceae) is oldest herbal healing plant known to mankind. It consists of short stem with thick leaves which store water and can survive in drought for long time period. More than 100 components in *aloe vera* possess wound healing properties as well as anti-diabetic astringent, antiulcer, hemostatic, antiseptic, anti-inflammatory antibacterial, antioxidant, antidiarrheal and anticancer properties. *Aloe vera* fresh plant possess 96% water and remaining are essential oils, amino acids, minerals, vitamins, enzymes and glycoproteins. *Curcuma longa* or Turmeric (Zingiberaceae) is a perennial herb which contains curcumin (diferuloyl methane), turmerol or turmeric oil. Volatile oil obtained from turmeric also exhibits antibacterial and potent anti-inflammatory and analgesic activities. Turmeric also contains fats, protein and vitamins, which play important role in would healing. Curcumin has essential role in disorders like diabetic ulcers, rheumatism, biliary disorders, cough and sinusitis in Indian traditional medicine. Curcumin and lime paste has been a common remedy for the treatment of inflammation and wounds. Curcumin is present in 2 to 5 % in turmeric and is one of the three curcuminoids (Anamika, 2012). In more recent times, curcumin has been studied extensively for its use as an anti-aging (Lima et al., 2011), anti-cancer (Shehzad et al., 2013), and wound healing agent (Maheshwari et al., 2006). Juice obtained from the fresh rhizome is commonly applied to leech bites, bruises and wounds.

The present study is carried out to describe that *aloe vera* gel along with other natural traditional excipients is effective in wound healing. The increase in popularity of herbal and natural medications, cost-effectiveness, ease in availability of raw materials and paucity of adverse reaction, encouraged to formulate the polyherbal topical preparations to improve the wound healing activity.

II. MATERIAL & METHODS
A. Collection of plant materials
*Aloe vera* (common name: Aloe, traditional name: *Kanwar botti*), leaves and *curcuma longa* (common name: Turmeric, traditional name:...
Haldi) rhizomes were collected from Bio Park of Bahauddin Zakariya University Multan during month of February 2017. Both drugs were analyzed for phytochemicals from Department of Pharmacognosy, Faculty of Pharmacy, Bahauddin Zakariya University Multan.

B. Chemicals
All the chemicals were pure and of analytical grade; all were purchased from Merck Company (Germany). Nitrofurazone ointment was purchased from Glaxo Smith Kline.

C. Preparation of Herbal Extract
Full size mature and thick succulent leaves of aloe vera were collected, washed with distilled water, rind was removed and was cut transversely. Colorless parenchyma was obtained with vegetable peeler, grounded in a blender and was centrifuged at 10000 rpm for 30 min at 4±2ºC to remove the fibers. The supernatant was lyophilized and was stored in airtight jars at 4ºC until use in preparation of gel (Chithra et al., 1998).

Fresh rhizomes of turmeric were cleaned from extraneous material, washed with distilled water, dried under shade, then were powdered mechanically, weighed and finally stored in airtight jars. About 1-liter ethanol (95% v/v) was added to 250 g turmeric powder for 3 to 4 days. Mixture was stirred with sterile glass rod after 12 hrs. and was filtered with Whatman filter paper no 1 for 3 times. In rotary evaporator (Eyela Co. Ltd., Japan), solvent was removed under reduced pressure at temperature less than 50 ºC leaving mustard yellow residue which was stored in glass airtight jars at 4 ºC till use. Weight of extract was recorded and percentage yield was recorded 8.65% (16).

D. Formulation of topical Gels
Aloe vera gel powder and Carbopol-940 was dissolved in sufficient quantity of water and was kept overnight. Triethanolamine or sodium hydroxide 10% was added to this mixture and was stirred vigorously to form a gel and was place in a beaker. The beaker was placed on water bath and temperature was allowed to reach 50ºC. Weighed quantity of 1 and 3 gm of curcuma longa extract was added to the mixture to make Gel-I (1% w/w) and Gel-II (3% w/w) respectively. Weighed quantities of propyl and methyl paraben was added in water in another beaker and was heated to dissolve. Propylene glycol and polyethylene glycol was mixed in separate beaker and was added to first beaker. Remaining quantity of purified water was added and pH was adjusted with triethanolamine or 10 % sodium hydroxide solution. Gels were packed in airtight jars. The herbal extract was incorporated into the formulation in two different concentrations. Composition of formulation is given in table 1.

Table 1. Development of gel formulation

<table>
<thead>
<tr>
<th>Ingredients percentage</th>
<th>Gel-I</th>
<th>Gel-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera gel powder % w/w</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Curcuma longa (Turmeric) % w/w</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Carbopol-940</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Propylene glycol 200</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Triethanolamine/Sodium Hydroxide 10 %</td>
<td>Quantity sufficient to neutralize the gel base</td>
<td>Quantity sufficient to neutralize the gel base</td>
</tr>
<tr>
<td>Purified water</td>
<td>q. s to make 100 gram</td>
<td>q. s to make 100 gram</td>
</tr>
</tbody>
</table>
E. Evaluation of Gels

Physical Stability:
Gel formulations were evaluated for physical parameters like color, odor, consistency, transparency and homogeneity. Gels were packed in plastic tubes and placed in the stability chamber and kept at 40º C, 37º C and at room temperature with 75% relative humidity for three months and were periodically evaluated for appearance of any undesirable color or odor.

Spreadability:
0.5 gm of gels were placed on glass slide in 1cm circle of on glass slide, over which another glass slide was placed. 125g of weight was placed for 5 min. Due to spreadability, the increase in diameter was noted (Joshi and Patravale, 2006).

Viscosity:
Viscosity of gels was measured with the help of Brookfield DV-E viscometer (RVDVE). The spindle (# 07) was inserted in test formulation and was sheared at 20, 60 and 100 rpm at room temperature. Distilled water was used to prepare gel formulations (Vador et al., 2012).

pH:
pH of gels was measured with pH meter (Transmark i.s 126). 0.5 gm of gels dissolved in 50 ml of distilled water was stored for 2 hrs. pH values were taken thrice and results of average values given in table-2. (Panigrahi et al., 1997, Derle et al., 2006).

Extrudability:
A simple method was implemented for this study. All formulations were packed in the plastic tubes after setting in container. Extrudability of all gels was evaluated with respect to weight in grams which is required to extrude about 1 cm of gel ribbon in 20 second (Kim et al., 2003).

F. Pharmacological studies

Experimental Animals:
Albino rabbits having weight range between 1 to 1.5 kg were placed in poly propylene cages (2 animals per cage) which were maintained at standard conditions (12/12 hr. light/dark cycle; 23±1 ºC, 35- 60% RH). They were fed on standard water ad libitum. The animals had access to water and food. The animals were trained to laboratory condition for 48 hrs. before experimental protocol in order to minimize non-specific stress. Experimental protocol was approved by the Institutional Animal Ethical Committee letter no 014/PHP/EC-17 dated 07/10/2017. The animals were placed in four groups, each group having 6 animals. Group I considered control and received no treatment, animals in group II received standard drug like Nitrofurazone, animals in group III received gel I, containing 1% w/w curcuma longa extract along with 3% w/w extract of aloe vera and animals in IV group received gel II containing 3% w/w curcuma longa extract along with 3% w/w aloe vera extract. Animals did not receive any other medications throughout the study. Animals having any infection or wound deterioration were removed from study and fresh animals were used in its place.

G. Skin irritation test:
The irritation test was carried out on shaved back of rabbit skin. 50 mg of the each gel was rubbed over 1cm square of naked skin of rabbit and was observed for any signs of edema and erythema (Marzulli and Maibach, 1997).

H. Wound creation

Excision wound model:
Rabbits have excision wounds explained by Malon and Morton (Morton and Malone, 1972). Rabbits were anaesthetized before wound creation, with about 0.2 ml I/V Lignocaine HCl 2% (Barrett Hodgson) about 4 mg/kg body weight. Animals were shaved at dorsal fur with the help of an electric clipper and wound area created was marked with methylene blue on the back of the animals using sterile circular stencil. Excision wound of 2.5 cm thickness was created with a surgical scissor to the depth of subcutaneous tissues in sterile conditions. All animals were placed in separate cages. First day of wound creation was considering as zero day. The percentage of wound contraction was considered as reduction in wound area and was recorded on 0, 3, 6, 9 and 12 days. Wound area was measured after tracing with a graph paper
in a planimetric manner. Period of epithelialization was obtained when the scar was removed and there was no residual raw wound. (Table 3). Gel was applied on excision wound once a day for 12 days.

**Incision wound model:**
Rabbits were anaesthetized using 0.2 ml I/V Lignocaine HCl 2% (Barrett Hodgson) about 4 mg/kg body weight. About 5 cm long paravertebral incision at a distance of 1 cm was created on cutaneous surface of rabbit. Wound was sutured using curved needle (No.10) and surgical nylon. On 7th day, sutures were removed and on 19th day, tensile strength was noted. (Lee, 1968).

**I. Assessment of wound healing**
The percentage of reduction in wound size determines rate of wound contraction on every day. Wounds were covered with gel and bamdage and were examined to check the size of wound contraction. Following Eq. was used for calculating wound contraction (Gong et al., 2013).

\[ \text{Wound contraction} (\%) = 100 \times \frac{(\text{first day wound size} - \text{wound size on specific day})}{\text{first day wound size}} \]  

**J. Histopathological studies**
Under light anaesthesia application, skin samples were taken on 0, 5th and 12th day for histopathological studies. 10 % buffered formalin was used for fixing tissues which were passed through alcohol of different grades and were fixed in paraffin wax. Tissue sections were stained with eosin and hematoxylin. Keratinization, fibroblast proliferation, neovascularization, epithelialization and angiogenesis was examined under microscope (Labomed LB-202, USA) (Yeo et al., 2000)

**K. Statistical analysis**
In this study, the difference between treatment and control groups was checked for its significance and student t-test was applied on data. When \( P<0.05 \), differences were significant.

**III. RESULTS**

**A. Evaluation of gel**
Both formulations were pale yellow in color and the physical evaluations were given in Table 2. Formulations were found neutral with pH range of 6 to 7. Spreadability represents area to which the gel spreads after application to affected part or the skin. Efficiency of a gel in terms of bioavailability depends on the spreading value. The extrudability represents the capacity of gel formulation to get out of tubes in uniform manner while squeezing the tube. The results of extrudability and viscosity are given in table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gel-I</th>
<th>Gel-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Translucent, Greasy, Transparent, Yellow, Homogenous</td>
<td>Translucent, Greasy, Pale Yellow, Homogenous</td>
</tr>
<tr>
<td>pH</td>
<td>6.88</td>
<td>6.49</td>
</tr>
<tr>
<td>Spreadability</td>
<td>35mm</td>
<td>32mm</td>
</tr>
<tr>
<td>Viscosity</td>
<td>53400cps</td>
<td>76200cps</td>
</tr>
<tr>
<td>Extrudability</td>
<td>Excellent</td>
<td>Good</td>
</tr>
<tr>
<td>Stability (pH, viscosity, spreadability)</td>
<td>1st Month +</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd Month +</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3rd Month +</td>
<td>+</td>
</tr>
</tbody>
</table>
B. Wound healing study

Wound contraction indicates reduction of wound area. Table 3 shows percentage of wound contraction in all groups for 12 days. It was observed that wound contraction was greater when treated with gel II and was significantly greater (p<0.05), complete healing was observed within 12 days in this group. Rate of wound contraction was least in control group, which receives no treatment. Standard formulation which was received from market showed better healing than gel I. It was observed that gel II containing 3% w/w curcuma extract showed better healing as compare to gel I containing 1% w/w curcuma extract. Table 3 shows the effect of curcuma longa extract 1% w/w and 3 % w/w along with 3% w/w aloe vera extract in wound healing. Treated animals were found to epithelialize in 11 and 9 days with 1 % w/w and 3 % w/w respectively. While the control and those treated with standard gel epithelized in 16 and 12 days respectively. After 12 th day, the percentage of reduction in wound area in standard, control, gel I and gel II treated rabbits was 56.32%, 81.15%, 86.21 and 100% respectively and the differences were statistically significant (P<0.05).

Table 3. The effect of formulations on excision wound healing in rabbits.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wound contraction in % age</th>
<th>Epithelization time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day</td>
<td>3rd Day</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>8.32±1.20</td>
</tr>
<tr>
<td>Standard</td>
<td>0</td>
<td>22.80±2.01</td>
</tr>
<tr>
<td>Gel-I</td>
<td>0</td>
<td>31.26±1.43</td>
</tr>
<tr>
<td>Gel-II</td>
<td>0</td>
<td>43.29±1.51</td>
</tr>
</tbody>
</table>

Note: All values are mean ± Standard Deviation of six animals in each group. All values are significant at p<0.05 vs Control; Gel-I: containing 1%w/w curcuma longa extract +3%w/w aloe vera extract; Gel-II: containing 3%w/w curcuma longa extract +3%w/w aloe vera extract

C. Histopathological study

Histopathological studies reveal the suitability of herbal products in wound healing (Table 4). Microscopic images of skin samples shown in Fig. 1 and 2 for 5th and 12th day of post wounding respectively.

5th day sampling: Fig. 1A was photomicrograph of animal skin which received no treatment (control) with excessive inflammatory cells. In this section, no epithelial layer was found and neutrophils started gathering around blood vessels. Fig. 1B was photomicrograph of section taken from animal treated with standard gel showed connective tissues which were well established, re-epithelialization was seen in some areas, neutrophils gathered around blood vessels which further resulted in angiogenesis. Fig. 1C was photomicrograph of section taken from animal treated with Gel I containing 1% w/w C. longa extract along with 3% w/w aloe vera leaf
extract, here blood vessels were well organized, epidermis was also observed showing re-epithelialization, less no of fibroblasts were observed in this wound. Fig. 1D was photomicrograph of section taken from animal treated with Gel II, containing 3% w/w C. longa extract along with 3% w/w aloe vera leaf extract, macrophages were observed but were less in number as compare to control group, connective tissues were also present, all layers of epidermis were well established showing faster re-epithelialization.

**Figure 1: Microscopic images of wounds taken on 5th day of treatment in rabbits.** A) Control skin: excessive inflammatory cell, no epithelial layer, neutrophils around blood vessels. B) Standard: well established connective tissues, neutrophils around blood vessels. C) Gel I: well organized blood vessels and epidermis, less number of fibroblasts. D) Gel II: less established macrophages, but well established epidermis, connective tissues and blood capillaries were present.

**12th day sampling:** Fig. 2A was photomicrograph of animal skin which received no treatment (control) which showed low number of neutrophils and neovascularization was very slow as number of blood vessels are less in number. Fig. 2B was photomicrograph of animal skin treated with standard gel which showed prominent epidermis and faster rate of epithelialization, number of neutrophils and fibroblasts were also developed, hair follicles were also seen. Fig. 2C was photomicrograph of section of animal treated with Gel I containing 1% w/w C. longa extract along with 3% w/w aloe vera leaf extract, all layers of epidermis were intact and re-epithelialization was faster, macrophages were abundant in numbers. Fig. 2D was photomicrograph of section of animal treated with Gel II containing 3% w/w C. longa extract along with 3% w/w aloe vera leaf extract, showing presence of macrophages and...
fibroblasts which were good in number and gathered around blood vessels. All layers of epidermis were well established and in proper shape.

Figure 2: Microscopic images of wounds taken on 12\textsuperscript{th} day of treatment in rabbits. 2A) Control skin: less number of neutrophils and blood vessels. 2B) Standard: prominent epidermis, neutrophils and developed fibroblasts. 2C) Gel I: intact epidermis layers, abundant macrophages. 2D) Gel II: macrophages present, good number of fibroblasts around blood vessels, well established epidermal layers.

Table 4. Histological evaluation of wounds treated with herbal gel at 12\textsuperscript{th} day along with measurement of tensile strength

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Keratinization</th>
<th>Fibrosis</th>
<th>Collagen</th>
<th>Epithelialization</th>
<th>Neovascularization</th>
<th>Tensile strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2±0.12</td>
<td>2.1±0.23</td>
<td>2.6±0.39</td>
<td>1.6±0.29</td>
<td>0.6±0.30</td>
<td>268.0±11.50</td>
</tr>
<tr>
<td>Standard</td>
<td>3.9±0.16</td>
<td>3.7±0.29</td>
<td>3.8±0.33</td>
<td>3.8±0.23</td>
<td>4.1±0.26</td>
<td>340.0±15.54</td>
</tr>
<tr>
<td>Gel-I</td>
<td>3.8±0.12</td>
<td>3.8±0.21</td>
<td>3.9±0.51</td>
<td>3.9±0.11</td>
<td>4.0±0.23</td>
<td>335±12.65</td>
</tr>
<tr>
<td>Gel-II</td>
<td>4.0±0.14</td>
<td>3.9±0.12</td>
<td>4.0±0.19</td>
<td>3.9±0.13</td>
<td>4.1±0.24</td>
<td>350±8.29</td>
</tr>
</tbody>
</table>

Note: All values are mean ± Standard Deviation of six animals in each group. All values are significant at p<0.05 vs Control. 5 value shows more resemblance and 0 shows less resemblance of wound with normal skin tissue.
IV. CONCLUSION

Objective of this study was to evaluate that herbal gels help in wound healing and provide protection against microorganisms. Majority of medicinal plants have beneficial role in wound healing (Wild et al., 2010). In this study, combination aloe vera and curcuma longa in 1:3 and 3:3 have been used in the form of gel for wound healing. These two gels provide wound healing effects on both excision and incision wounds. Results showed that gel II containing aloe vera 3% w/w along with curcuma longa 3%w/w had better wound healing on 5th day of treatment but there was no major difference in wound contraction study in gel I, gel II and standard gel till 16th day. Likewise, in histopathological studies, re-epithelialization was better in animals treated with gel II as compare to other groups. Number of blood capillaries in gel II treated animals was also greater than in other groups, presence of blood vessels or capillaries showed neovascularization as well as indicative of wound healing. Tensile strength of wound treated with gel II increased because of increasing strength of fibers and collagen concentration.

Wound healing plants possess antimicrobial, anti-inflammatory and antioxidant properties and these properties contribute in formulation of new products for wound healing (Gong et al., 2013, Jahandideh et al., 2017). According to reports, aloe vera increases collagen contents which helps in cross linkage of collagen and proliferation of fibroblasts, increases angiogenesis and results in production of growth factors (Abdallah et al., 2009). Several researchers evaluated that the biological activity of polysaccharides (glucomannan, acemannan, pectic acid, mannose-6-phosphate and galactan) and glycoproteins (lectins) found in leaf pulp, play important role in process of the wound-healing. (Hashemi et al., 2015, Salazar-Sánchez et al., 2010, Ramachandra and Rao, 2008). Aloe vera gel enhances wound closure and wound contraction. In the late stages of the wound healing, aloe vera gel increases neovascularization, epithelialization, and increases wound contraction. The mucilage of aloe vera also increases transversal connections among collagen bands without changing collagen structure, therefore accelerates wound improvement (Hashemi et al., 2015). Rhizomes of curcuma longa contain curcumin (diferuloyl methane), turmerol or turmeric oil exhibit antibacterial, potent analgesic as well as anti-inflammatory and activities. Protein, fats, vitamins (A, B, C etc.) present in curcuma longa, have important role in wound healing, regeneration process and in synthesis of collagen fibers by mimicking activity fibroblast. (Rao et al., 2003). Therefore, gel containing aloe vera and curcuma longa could be suggested appropriate for wound healing because of the constituents in these herbs.

From the findings, it was concluded that the formulations were physically and chemically stable at 40ºC for 90 days. Gel I was more superior in spreadability and texture as compare to Gel II. Wound healing activity of Gel II was higher than Gel I and that obtained from market. Histopathological studies showed that the process of neovascularization and collagen formation was better in tissues treated with Gel II. Wound healing studies also revealed that wound treated with Gel II contracted in 12 days only while others took extra time. It was found that Gel II containing 3%w/w C. longa extract in combination with 3% w/w aloe vera extract was more stable and more superior than other formulation.

V. REFERENCES

a review. The international journal of lower extremity wounds, 2, 25-39.


