CHAPTER IV

RESULTS

1. Characteristic feature of *Centella asiatica* (CA) extract

It has been reported that triterpenes i.e., asiaticoside, asiatic acid and madecassic acid, are the major active constituents in wound healing activity of CA (Farnsworth and Bunyapraphatsara, 1992; Bonte et al., 1993; Sunikumar et al., 1998; Maquart et al., 1999; Punturee et al., 2004; and Park et al., 2005). Furthermore, ß-sitosterol, another constituent in CA has been demonstrated to possess angiogenic activity, an essential factor in wound healing (Moon et al., 1999; Choi et al., 2002). In this study, different fractions of CA: hexane (HE), ethyl acetate (EE), methanol (ME) and water extract (WE) were used. Therefore, the major active compounds in each extract were identified by thin layer chromatography technique and the chromatograms were shown in figures 4.1, 4.2, 4.3 and 4.4.

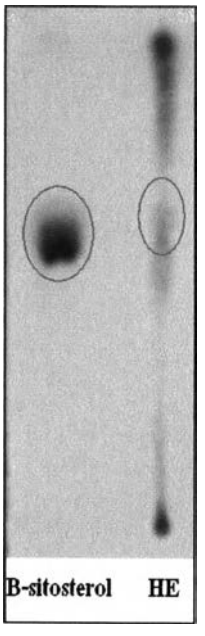

Figure 4.1 TLC of hexane extract of CA using ß-sitosterol as standard compound. The lane of HE exhibited a spot on TLC at the same position of ß-sitosterol.

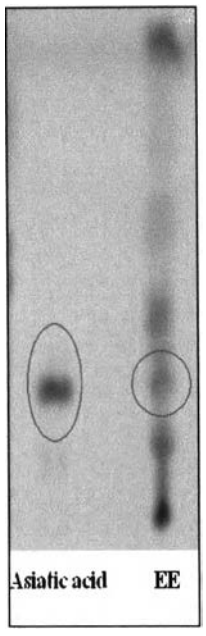

Figure 4.2 TLC of ethyl acetate extract of CA using asiatic acid as standard compound. The lane of EE exhibited a spot on TLC at the same position of asiatic acid.

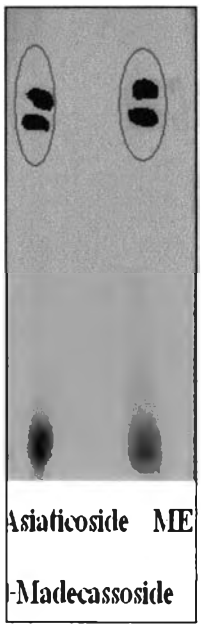

Figure 4.3 TLC of methanol extract of CA using asiaticoside and madecassoside as standard compounds.

The lane of ME exhibited two spots on TLC at the same position of asiaticoside (R_f=0.4) and madecassoside (R_f=0.5).

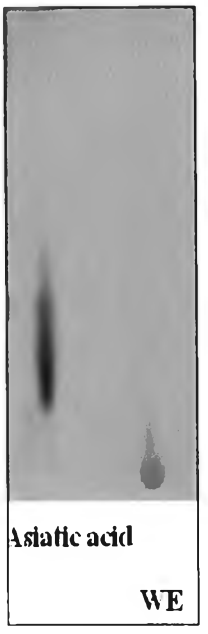

Figure 4.4 TLC of water extract of CA using asiatic acid as standard compound. The lane of WE exhibited no spot on TLC.

2. Effect of the *Centella asiatica* (CA) extracts on wound healing

2.1 Effect of the *Centella asiatica* (CA) extracts on healing of incision wound

Healing of incision wound was evaluated by a measurement of tensile strength on day 7 post wounding. The tensile strength of untreated animals (14.99 $\pm$ 1.72 N/cm^2) was not significantly different from those found in NSS-treated group (17.62 $\pm$ 1.42 N/cm^2) or Tween-20-treated group (TW) (14.69 $\pm$ 0.72 N/cm^2) which served as a vehicle control group (Table 4.1). In comparison to control, tensile strength in all animal groups treated with 10% of different fractions of CA were significantly increased to 21.20 $\pm$ 2.24, 21.26 $\pm$ 1.77, 19.72 $\pm$ 0.84 and 21.05 $\pm$ 1.90 N/cm^2 in hexane (HE), ethyl acetate (EE), methanol (ME), and water extract (WE)- treated groups, respectively (Figure 4.5). However, none of them was significantly different from those of NSS- treated group (Table 4.1).

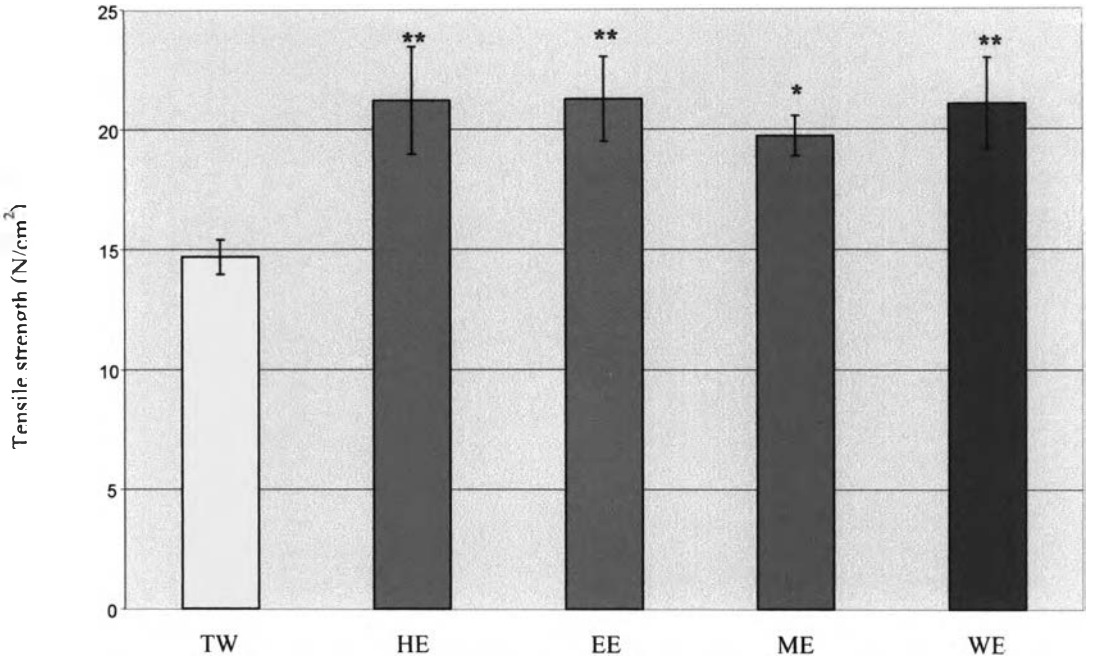

Figure 4.5 Tensile strength of wounds treated with Tween 20 solution and 10% of different fractions from CA extract at day 7 post wounding. Results are presented as means ± S.E.M., n =8, statistically significant results (in comparison to TW) are given as *, ** which represent values where $P<0.05$ and $P<0.01$, respectively.

Table 4.1 Tensile strength of incision wounds untreated and treated with NSS, Tween 20 or different fractions of CA extract at day 7 post wounding.

Results are presented as means ± S.E.M., n =8, statistically significant results (in comparison with TW) are given as *, ** which represent values where $P<0.05$ and $P<0.01$, respectively .

Group of treatment	**Tensile strength (N/cm^2)**
Untreated	14.99 ± 1.72
NSS	17.62 ± 1.42
Tween 20	14.69 ± 0.72
Hexane extract	21.20 ± 2.24**
Ethyl acetate extract	21.26 ± 1.77**
Methanol extract	19.72 ± 0.84*
Water extract	21.05 ± 1.90**

2.2 Effect of the *Centella asiatica* (CA) extracts on healing of burn wound

Efficacy of the extract from CA on burn wound was assessed by gross pathology evaluation (wound lesion and degree of wound healing) as well as histopathological observation.

2.2.1 Gross pathology evaluation

Gross pathological evaluation was made on day 3, 7, 10, and 14 after burning by an observation of wound lesion and an assessment of degree of wound healing.

2.2.1.1 Wound lesion

On day 3, the wound in untreated, NSS-treated and Tween 20-treated groups became swelling and bruised (Figure 4.6A, 4.6B, 4.6C, respectively). Comparatively, the wound in all extract-treated groups showed a mild degree of swelling and wound surface was rather dry (Figure 4.6D, 4.6E, 4.6F, 4.6G). Most wounds in ethyl acetate-treated group began to reduce in size by slightly contracting from the wound edge (Figure 4.6E). The wound appearances were illustrated in figure 4.6.

On day 7, all wounds in untreated, NSS-treated and Tween 20-treated groups showed dark red color, thickening of the skin at wound site and the wound sizes remain unchanged from the first day (Figure 4.7A, 4.7B, 4.7C, respectively). Most of the wounds treated with the extracts showed wound contraction, smaller in size compared with those in control groups (Figure 4.7D, 4.7E, 4.7F, 4.7G). Wounds in methanol extract-treated group showed remarkable hair growth (Figure 4.7F). Some wounds in the methanol extract- and water extract-treated group showed scabs covering the wound surface (Figure 4.7F, 4.7G, respectively).

On day 10, all wounds in untreated, NSS-treated and Tween 20-treated groups showed moderate exudation, no hair growth and wound surface was covered by scabs (Figure 4.8A, 4.8B, 4.8C, respectively). All wounds in the extract-treated groups showed dry wound surface, progressive wound contraction and reduction of wound sizes, and the hair growth was increased (Figure 4.8D, 4.8E, 4.8F, 4.8G).

On day 14, which was the end of the experiment, all wounds in untreated, NSS-treated and Tween 20-treated groups showed moderate exudation and scabs started to separate from the wound surface and the wound sizes were slightly decreased compared with those at the beginning (Figure 4.9A, 4.9B, 4.9C, respectively). The wounds in the extract-treated groups showed remarkable decrease in wound size and continuous growth of the hair at wound site (Figure 4.9D, 4.9E, 4.9F, 4.9G). The wound in methanol and water extract-

treated groups showed the most remarkable decrease in wound size (Figure 4.9F, 4.9G, respectively).

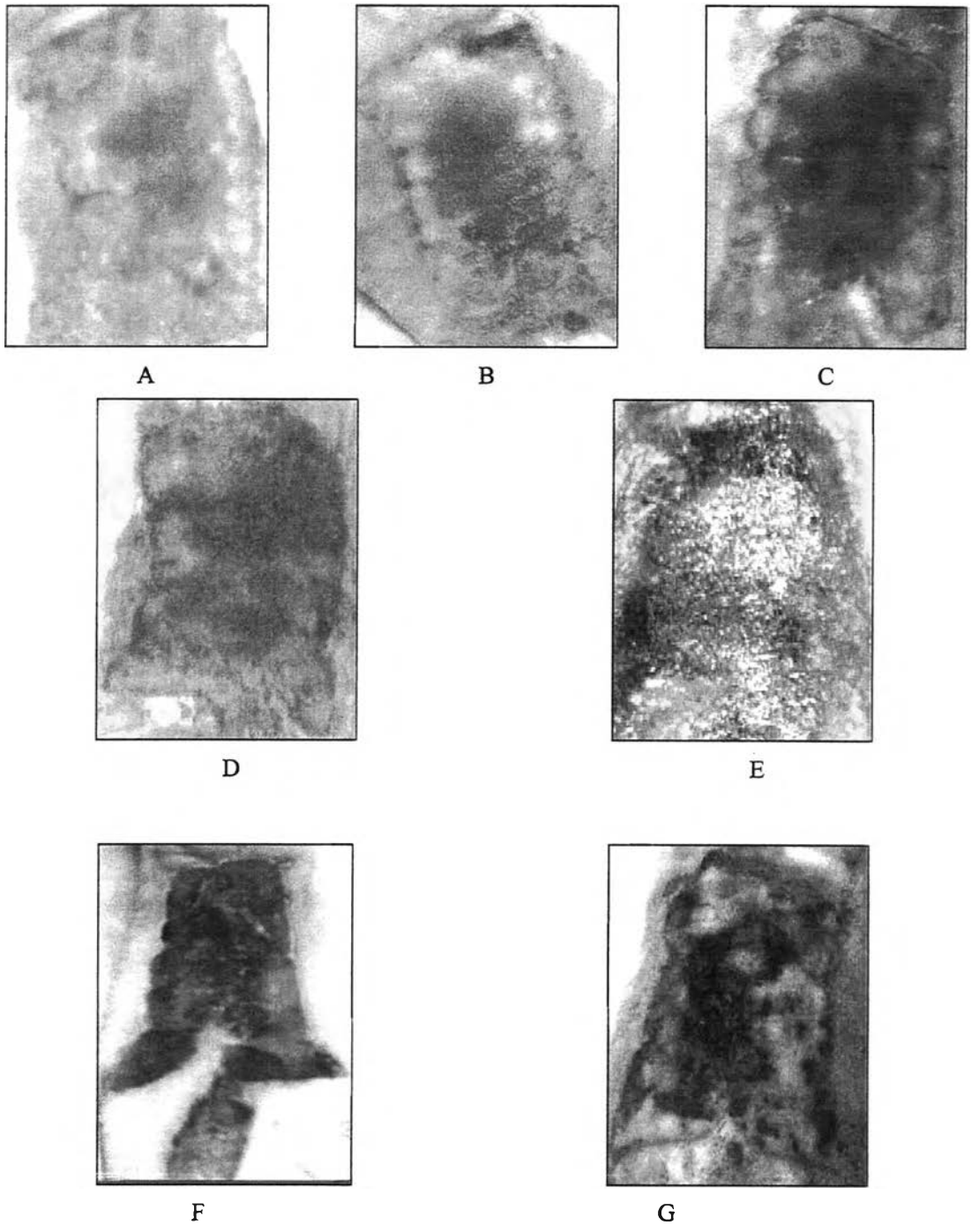

Figure 4.6 The wound appearance on day 3. A) Untreated wound B) NSS-treated wound C) Tween 20-treated wound D) Hexane extract-treated wound E) Ethyl acetate extract-treated wound F) Methanol extract-treated wound G) Water extract-treated wound

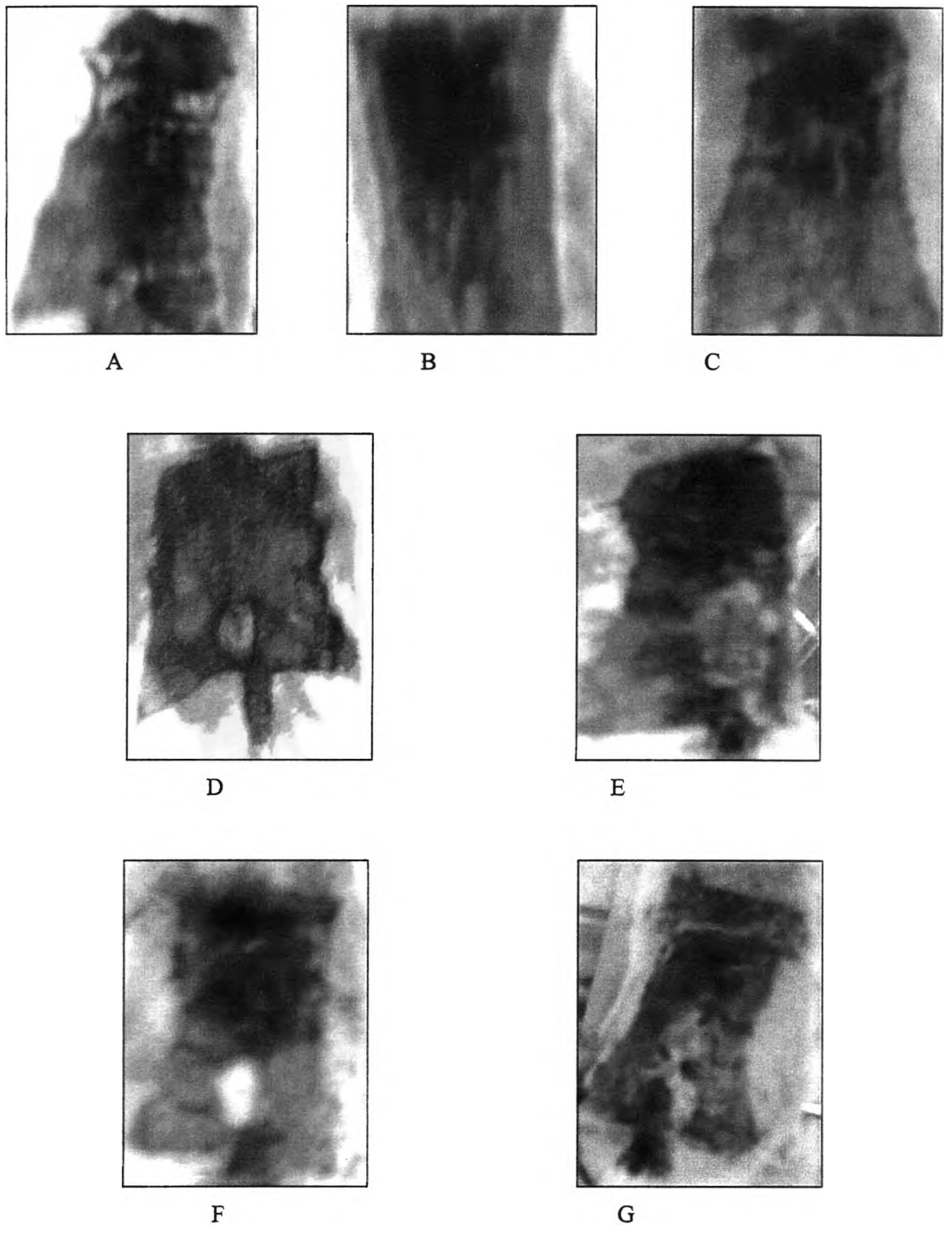

Figure 4.7 The wound appearance on day 7. A) Untreated wound B) NSS-treated wound C) Tween 20-treated wound D) Hexane extract-treated wound E) Ethyl acetate extract-treated wound F) Methanol extract-treated wound G) Water extract-treated wound

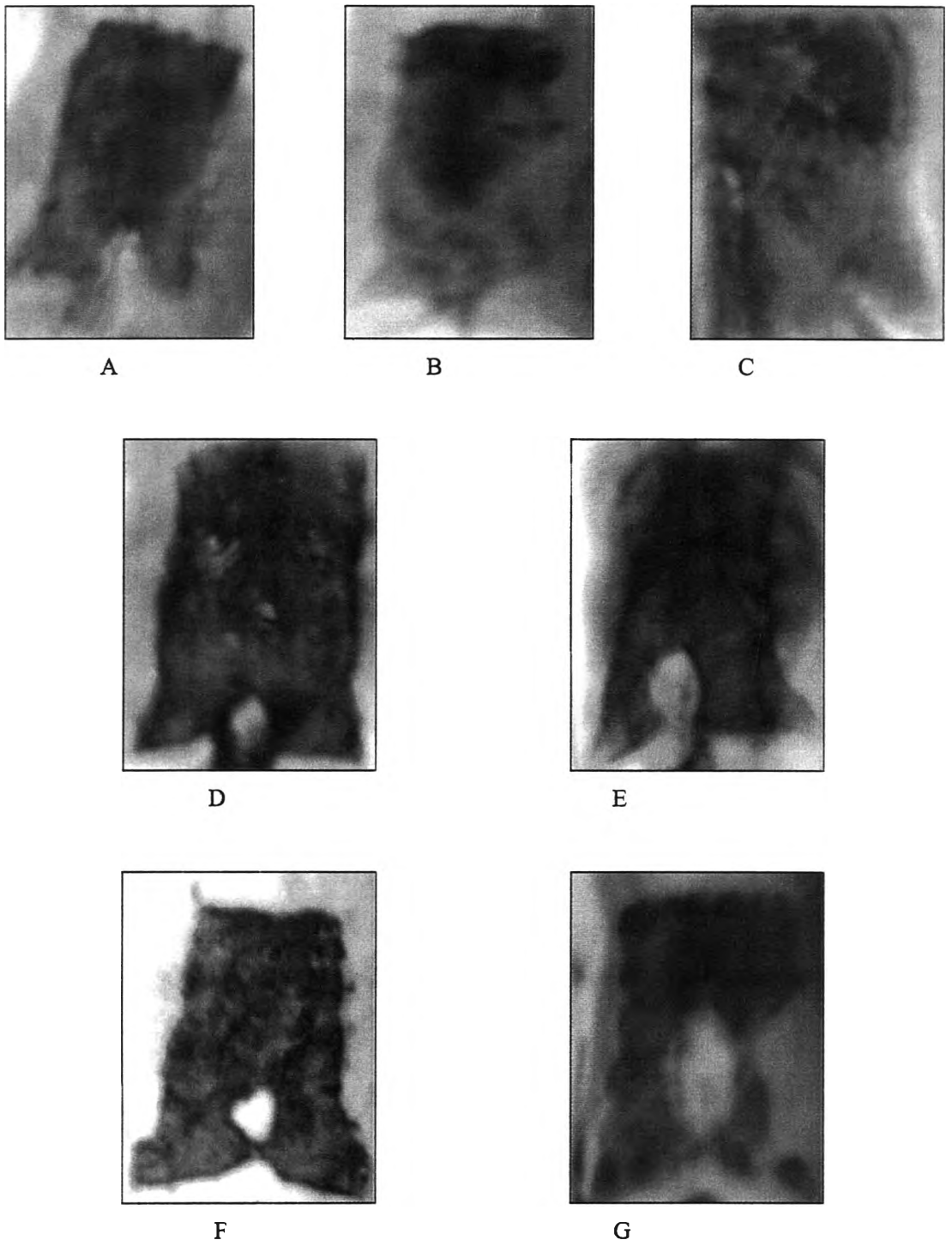

Figure 4.8 The wound appearance on day 10. A) Untreated wound B) NSS-treated wound C) Tween 20-treated wound D) Hexane extract-treated wound E) Ethyl acetate extract-treated wound F) Methanol extract-treated wound G) Water extract-treated wound

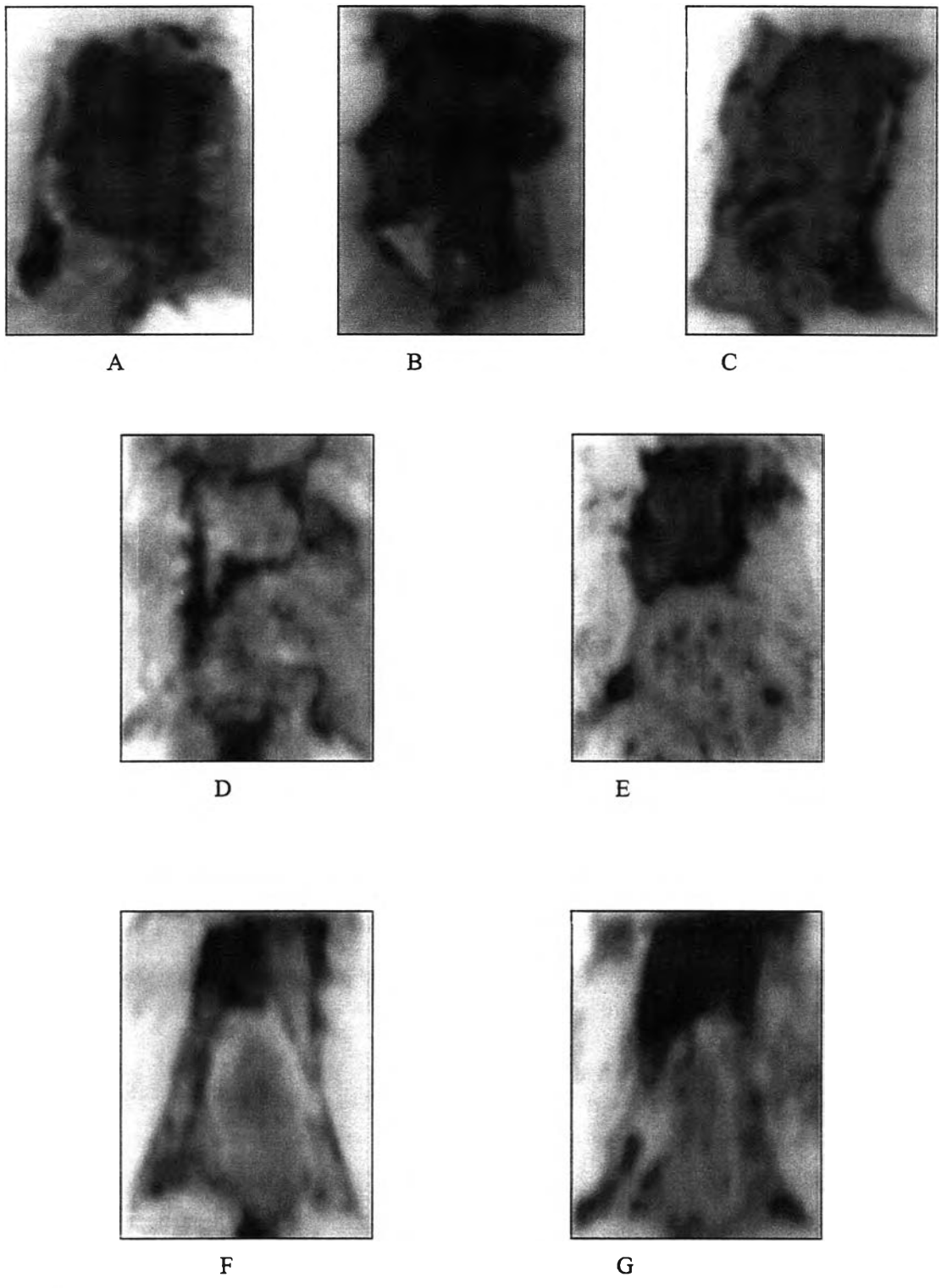

Figure 4.9 The wound appearance on day 14. A) Untreated wound B) NSS-treated wound C) Tween 20-treated wound D) Hexane extract-treated wound E) Ethyl acetate extract-treated wound F) Methanol extract-treated wound G) Water extract-treated wound

2.2.1.2 The degree of wound healing

The degree of wound healing was calculated on day 3 , 7, 10 and 14 post burning by using the formula previously described (Reddy et al., 2002).

Day 3 post burning:

On day 3 post burning, the degree of wound healing of untreated (9.51 ± 1.21%), NSS-treated (12.80 ± 2.65%) and Tween 20-treated group (10.05 ± 1.50%), were not significantly different from each other (Table 4.2), therefore Tween 20-treated group (TW) will be used as control for further comparison. In the extract-treated groups, only in ethyl acetate group that the degree of wound healing was significantly higher than those in vehicle control group (18.13 ± 2.29%)(Figure 4.10). In contrast, the degree of wound healing in the other three groups was not significantly different from those of Tween 20-treated group (11.12 ± 2.89%, 11.71 ± 1.94%, and 7.86 ± 1.45% in hexane, methanol and water extract, respectively.

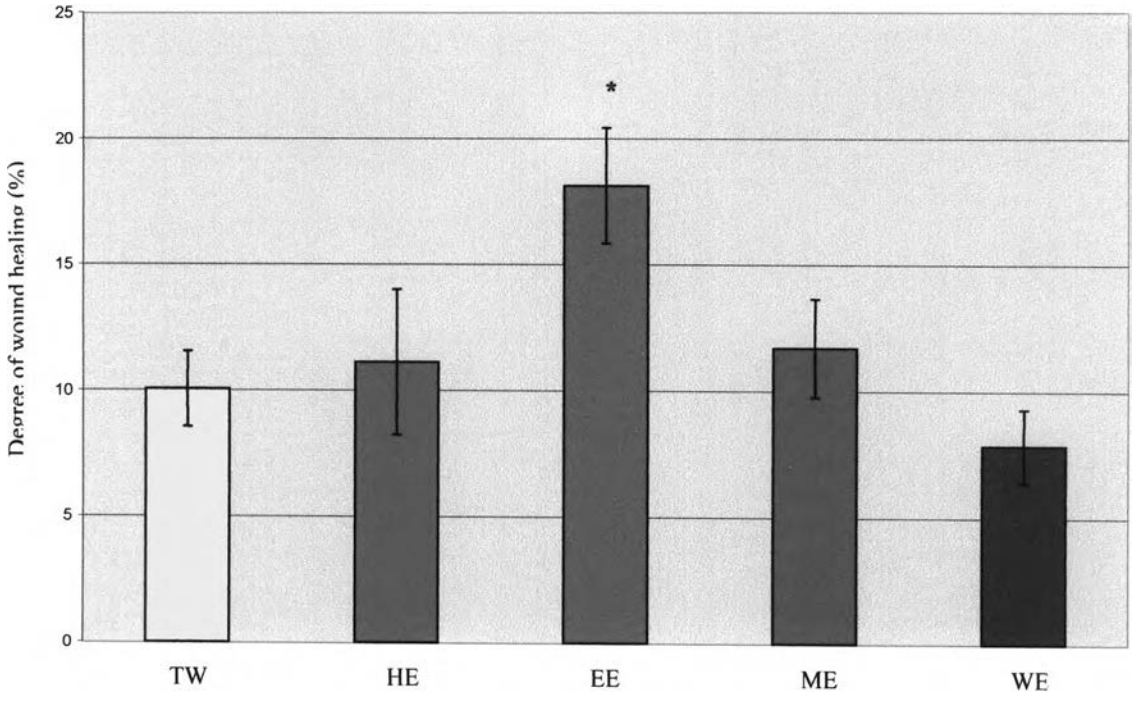

Figure 4.10 The degree of wound healing on day 3 post burning

Results are presented as means ± S.E.M., n = 8, statistically significant result (in comparison to TW) are given as * which represents value where $P<0.05$.

Day 7 post burning:

Similar results were observed on day 7 post burning. The degree of wound healing in the untreated animals (14.27 ± 2.10%) was not significantly different from those in NSS-treated (19.64 ± 2.34%) or Tween 20-treated groups (20.57 ± 2.58%) which served as control group (Table 4.2). Degree of wound healing in ethyl acetate extract-treated group (30.17 ± 1.81%) was significantly higher than those in vehicle control group (20.57 ± 2.58%)(Figure 4.11).

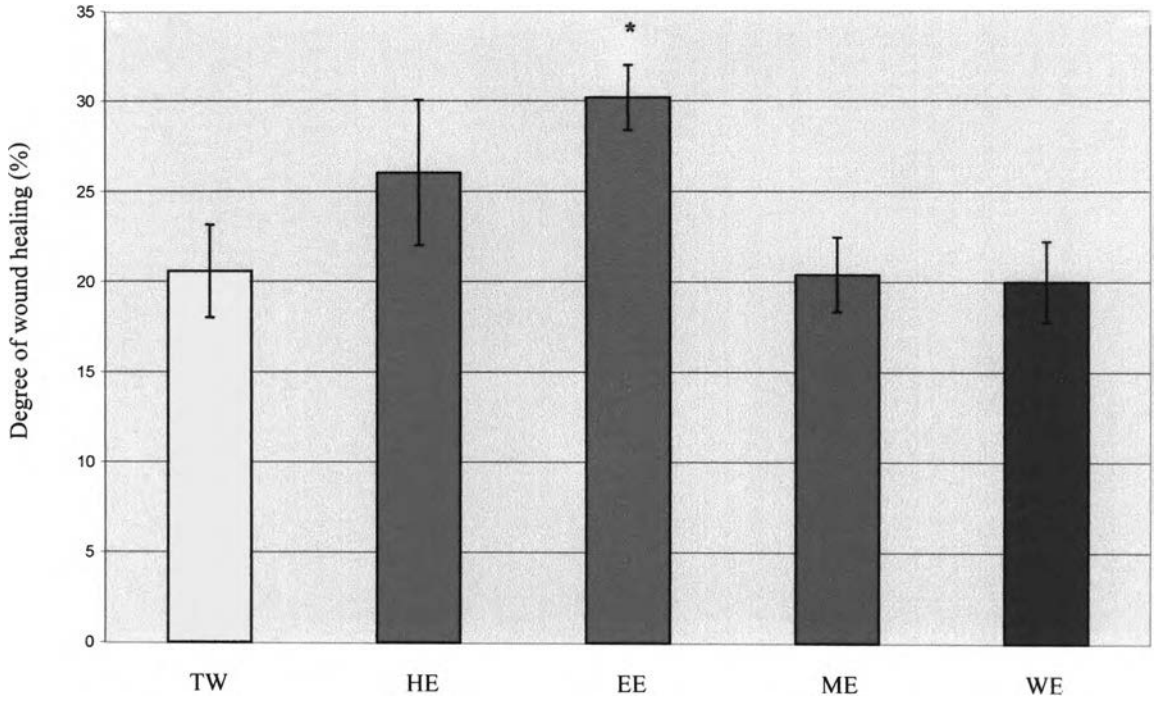

Figure 4.11 The degree of wound healing on day 7 post burning

Results are presented as means ± S.E.M., n = 8, statistically significant result (in comparison to TW) are given as * which represents value where $P<0.05$.

Day 10 post burning:

The degree of wound healing among the animals in untreated (20.21 ± 2.10%), NSS-treated (25.90 ± 2.67%) and Tween 20-treated groups (27.69 ± 2.39%), which served as a vehicle control group were not significantly different (Table 4.2). The degrees of wound healing in animals treated by hexane (37.78 + 3.89%) or ethyl acetate extract (38.11 + 2.30%) were significantly higher than those in control (27.69 ± 2.39%). Moreover, the degree of wound healing in hexane extract-treated group was not significantly different from those found in ethyl acetate extract-treated group (Figure 4.12).

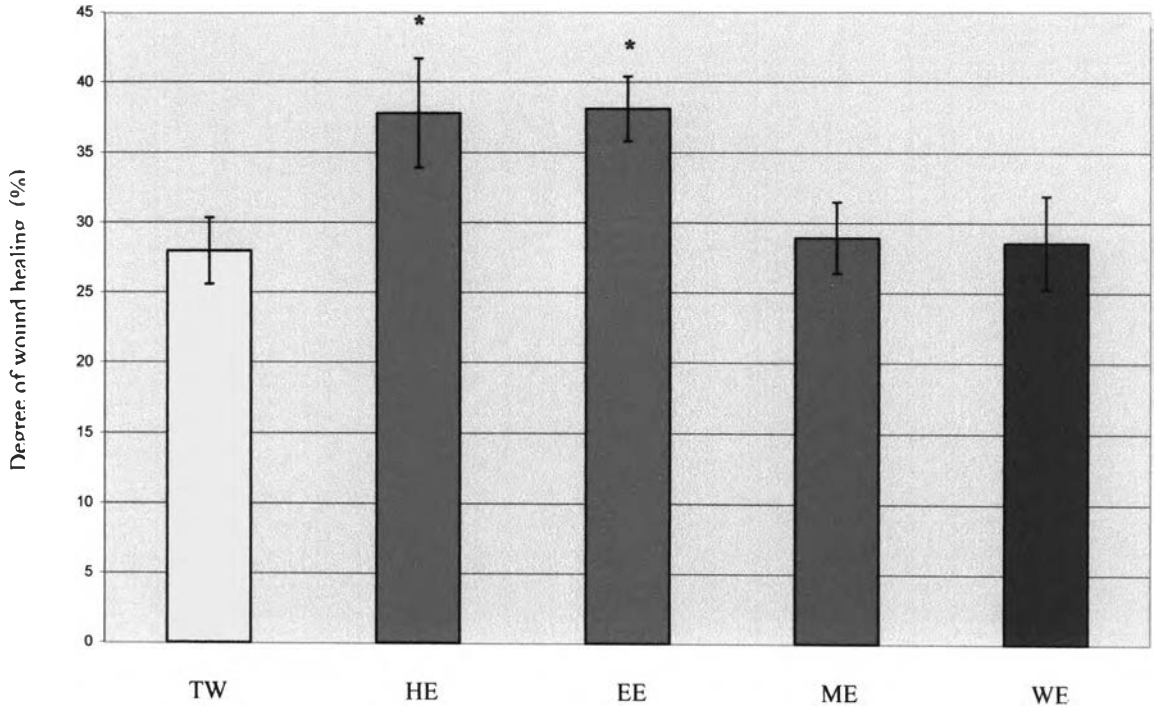

Figure 4.12 The degree of wound healing on day 10 post burning

Results are presented as means ± S.E.M., n = 8, statistically significant result (in comparison to TW) are given as * which represents value where $P<0.05$.

Day 14 post burning:

On day 14 post burning, the degree of wound healing in untreated (25.36 ± 1.81%), NSS-treated (31.85 ± 2.66%) and Tween 20-treated groups (38.07 ± 5.15%) were not significantly different from each other (Table 4.2). In contrast, the degree of wound healing in the animals from all extract-treated groups (53.87 ± 4.64%, 57.53 ± 5.68%, 60.31 ± 5.70%, and 59.82 ± 8.31% in hexane, ethyl acetate, methanol and water extract, respectively) were significantly higher than those in control group (38.07 ± 5.15%)(Figure 4.13). Furthermore, the degrees of wound healing among the extract-treated groups were not significantly different from each other (Figure 4.13).

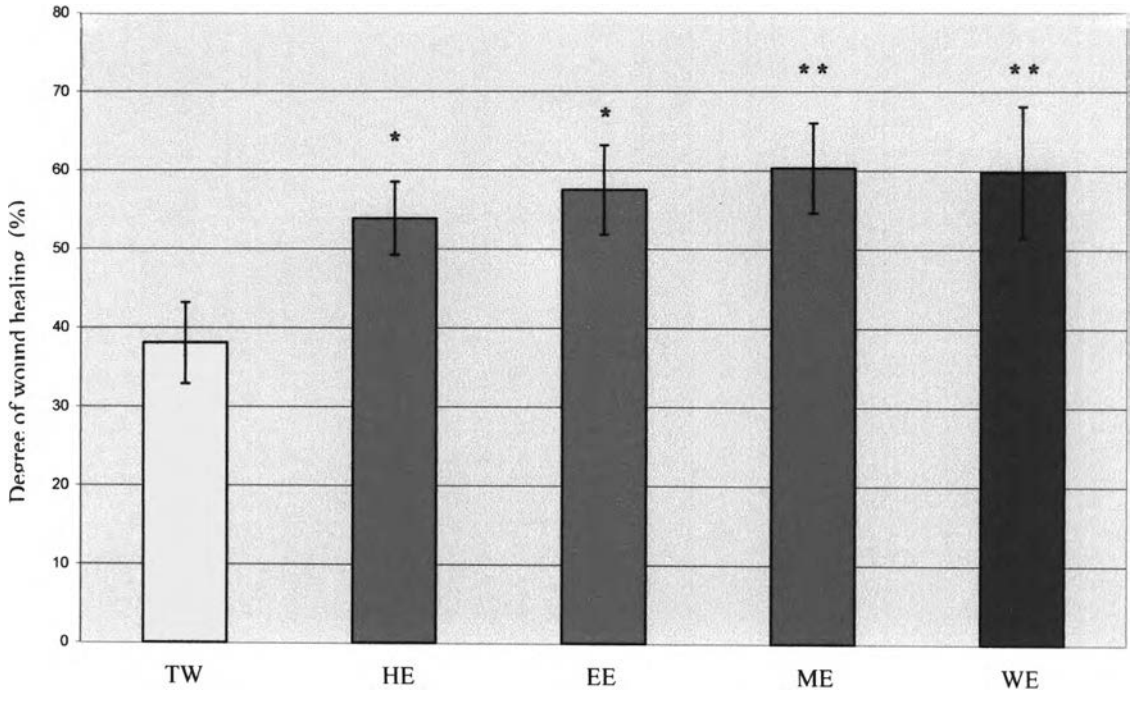

Figure 4.13 The degree of wound healing on day 14 post burning

Results are presented as means ± S.E.M., n = 8, statistically significant result (in comparison to TW) are given as * which represents value where $P<0.05$.

Table 4.2 The degree of wound healing in second-degree burn wound

Results are presented as means ± S.E.M., n = 8, statistically significant results (in comparison to TW) are given as *, ** which represent values where $P<0.05$ and $P<0.01$, respectively.

	Degree of wound healing (%)			
Group	***Day 3***	***Day 7***	***Day 10***	***Day 14***
Untreated	9.51 ± 1.21	14.27 ± 2.10	20.21 ± 2.10	25.36 ± 1.81
NSS-treated	12.80 ± 2.65	19.64 ± 2.34	25.90 ± 2.67	31.85 ± 2.66
TW	10.05 ± 1.50	20.57 ± 2.58	27.69 ± 2.39	38.07 ± 5.15
HE	11.12 ± 2.89	26.02 ± 4.02	37.78 ± 3.89*	53.87 ± 4.64*
EE	18.13 ± 2.29*	30.17 ± 1.81*	38.11 ± 2.30*	57.53 ± 5.68*
ME	11.71 ± 1.94	20.39 ± 2.07	28.94 ± 2.55	60.31 ± 5.70**
WE	7.86 ± 1.45	19.98 ± 2.23	28.57 ± 3.35	59.82 ± 8.31**

2.2 Histopathological observation

Histopathological evaluation of wound healing in this study was examined at the end of experiment on day 14 post burning (Figure 4.14, 4.15).

The untreated burn wound showed prominent fibrinoid necrosis in subepidermal region, which was characterized by permeation of collagen with fibrin and additional degenerative changes. The re-epithelialization was incomplete with less skin appendages. Wound surface was covered with some exudates (Figure 4.14B).

The wound in NSS- and Tween 20-treated groups showed fibrinoid necrosis in some areas of subepidermis. There were empty spaces in dermal region of Tween 20-treated group, as evidence of edema. Re-epithelialization was still incomplete but NSS-treated wounds seemed to be better than those treated with Tween 20 in this regard (Figure 4.14C, 4.14D, respectively).

The wounds in all extract- treated groups showed fully developed epithelialization and keratinization. Skin appendages can be observed near to normal skin. There were no noticeable necrosis and inflammation (Figure 4.15B, 4.15C, 4.15D, 4.15E).

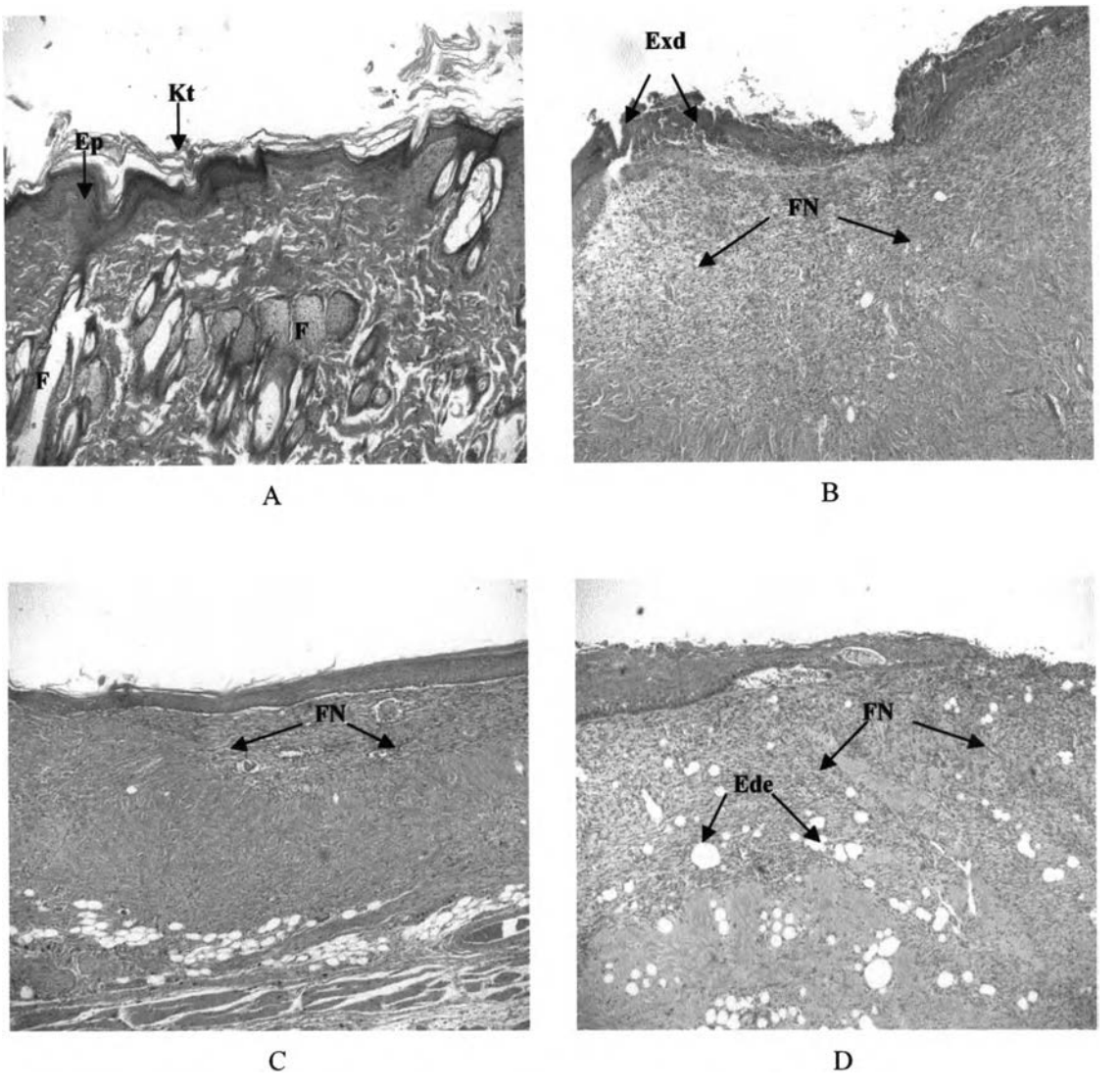

Figure 4.14 Hematoxylin-eosin stain. Histological change of skin section at day 14 post burning of A) normal skin, B) untreated wound, C) NSS-treated wound, D) Tween 20-treated wound; Ep = epidermis, Kt = keratin, F = hair follicle, Exd = exudates, FN = fibrinoid necrosis, Ede = edema (original magnitude x 10)

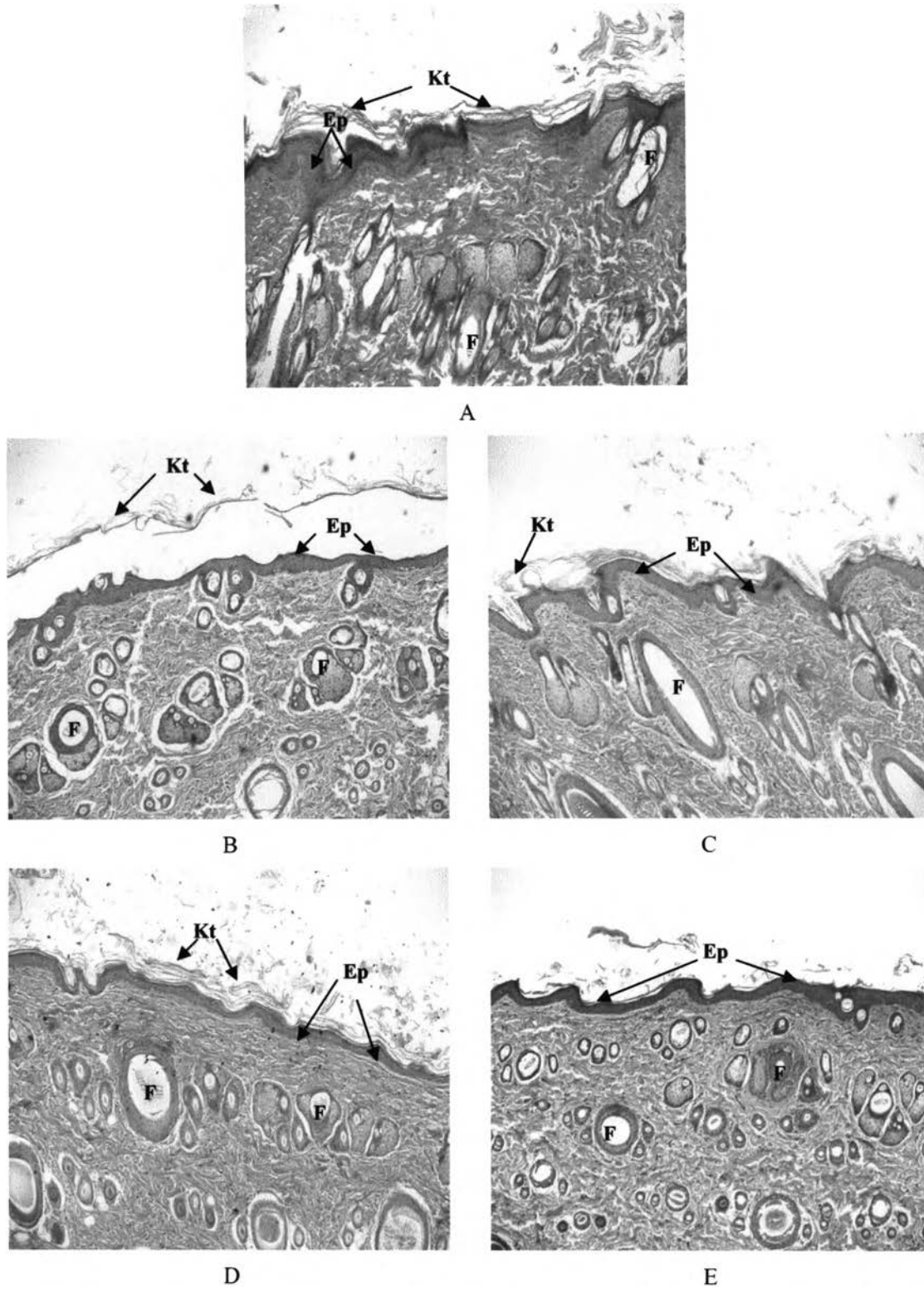

Figure 4.15 Hematoxylin-eosin stain. Histological change of skin section at day 14 post burning of A) normal skin, B) hexane extract-treated wound, C) ethyl acetate extract-treated wound, D) methanol extract-treated wound, E) water extract-treated wound; Ep=epidermis, Kt=keratin, F=hair follicle (original magnitude x 10)