Pharmacologia 3 (6): 160-168, 2012
ISSN 2044-4648 / DOI: 10.5567/pharmacologia.2012.160.168

Wound Healing Activity of *Clitoria ternatea* L. In Experimental Animal Models

Y.B. Solanki and S.M. Jain
Department of Pharmacology, L.M. College of Pharmacy, Navrangpura, Ahmedabad-380 009, India

Abstract: Background: *Clitoria ternatea* is important ayurvedic medicinal plant known as Butterfly pea and reported to have nootropic, anxiolytic, tranquilizing, anti-inflammatory, analgesic, antipyretic, antimicrobial and immunomodulatory activities. Present study was undertaken to investigate the wound healing activity of *C. ternatea* seed and root extracts. The effects on wound healing were investigated using excision, incision and dead-space models in rats. The extracts were standardized by HPTLC method. **Results:** *C. ternatea* seed and root extracts significantly improved wound healing in excision, incision and dead-space models when administered orally by gavage as well as applied topically as ointment. These effects are comparable to that of cotrimoxazole ointment. The finding of the study suggested that *C. ternatea* affects all three phases-inflammatory, proliferative and remodeling phases of wound healing. Plant extracts were found to contain phenolic compounds and seed extract was containing flavonol glycosides. **Conclusion:** The present study demonstrated wound healing activity of *C. ternatea* in animal models. The flavonol glycoside and phenolic compounds present in plant may be responsible for altering the inflammatory and immune component of wound healing.

Kay words: Aparajita butterfly pea, flavonoids, phenolic, wound healing

INTRODUCTION

Wound healing is a complex and highly dynamic process that rapidly close a wound and prevents infection after injury. It can be divided into inflammatory, proliferative and remodeling phases that involve numerous different cell types (Gurtner *et al*., 2008). In an ideal world, repair would result in regeneration of the original tissue with structural, functional and aesthetic attributes similar to that of uninjured skin. However, in normal wound healing process, the structural integrity is maintained by the replacement of damaged tissue with fibrotic material, leading to scarring (Stramer *et al*., 2007). Wounds like foetal skin wounds and oral mucosal wound repair more rapidly without scarring (Schrementi *et al*., 2008) due to limited inflammatory response during the repair (Eming *et al*., 2007). In wounds having robust inflammatory response like, diabetic ulcers, the healing is relatively slow (Martin and Leibovich, 2005). Thus, inflammation plays crucial role in the wound healing.

Upon injury, platelets are the first blood cells on the scene; that secrete biologically active proteins that bind to the fibrin mesh and to the ECM, creating chemotactic gradients that trigger the inflammatory phase of repair by recruiting immune cells to the wound (Martin and Leibovich, 2005). Neutrophils are the first nucleated immune cell to infiltrate a wound, acting as a first line of defense by decontaminating the wound (Dovi *et al*., 2004). Usually neutrophil infiltration ceases after a few days and the act of phagocytosis results in the neutrophil committing suicide by apoptosis. Around 48 h after the initial injury, monocytes are recruited via the numerous chemoattractants, including growth factors, cytokines and chemokines, produced by platelets, neutrophils, keratinocytes and fibroblast at the site of injury. During the repair process, macrophages are thought to play a pivotal role in fibrosis and scarring (Martin *et al*., 2003). Immune cells dictate the quality and speed of tissue repair (Rajan and Murray, 2008). Whenever the body's natural healing process is deregulated and wounds fail to progress through the typical orderly sequence of repair in a timely fashion, wounds heal with difficulties. Disruption of one or more of the healing stages can result in prolonged and incomplete repair, with lack of restoration of integrity. Non-healing wounds are a significant problem for healthcare systems all over the world. These wounds can cause significant pain and suffering, loss of independence and often interfere with quality of life.

Clitoria ternatea Linn. (family: Fabaceae), is popularly known as a "Butterfly pea" in western countries and as Aparajita in the traditional Ayurvedic system of medicine (Anonymous, 2003). It is reported to have brain tonic activity and is popularly known as shankhapushpi (Upadhye and Kumbhojkar, 1993) in India. In traditional

Corresponding Author: Y.B. Solanki, Department of Pharmacology, L.M. College of Pharmacy, Navrangpura, Ahmedabad-380 009, India Tel: +91-79-2630 2746 Fax: +91-79-6444865

system of medicine, it is employed against different disease conditions such as cathartic, purgative, demulcent, emetic and anti-inflammatory in swollen joints (Kirtikar and Basu, 1976; Chopra, *et al*., 1956). Ayurvedic system prescribed various part of the plant in inflammation, hepatic disorders and as a brain tonic (Anonymous, 2003). Various parts of *C. ternatea* (CT) have been reported to have nootropic activity, anxiolytic activity, tranquilizing property, anti-inflammatory and analgesic activity, antipyretic, antimicrobial activity and immunomodulatory activities (Mukherjee *et al*., 2008). The plant is found to possess antibacterial activity (Malabadi *et al*., 2005). The flavonol glycoside present in roots is reported to have antibacterial activity (Yadava and Verma, 2003). *C. ternatea* has been reported to have anti-inflammatory, hepatoprotective (Solanki and Jain, 2011), antihyperlipidemic (Solanki and Jain, 2010a) and immunoinhibitory activities (Solanki and Jain, 2010b). Therefore, it is worthwhile to evaluate effects of *C. ternatea* on would healing. Hence, the present study was conducted to evaluate an anti-inflammatory activity of seeds and roots of *C. ternatea* in experimental animals.

MATERIALS AND METHODS

Plant collection and identification: The plant is available in two varieties-blue one and white one. It is climbing vine found on road side and field sides throughout India. Since, the blue variety is medicinally more important, we used only blue Variety for the present investigation. The plant was collected in the month of March, 2007 from the fields and road side of the Charotar region of the Gujarat state, India. The pods were allowed to dry sufficiently under shade and finally seeds were collected manually.

The plant was botanically identified by Dr. G.C. Jadeja, Professor and Head of Agricultural Botany Department, B. A. College of Agriculture Anand Agricultural University, Anand, India. The specimens of the sample were stored in the museum of the department (specimen No. 0701). The quality of plant was ascertained as per Ayurvedic Pharmacopoeia of India by determining foreign matters, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive values (Anonymous, 2003).

Extraction: The dry powdered seeds (1 kg) were extracted with ethyl acetate by percolation until the percolate was free of green color. The residues were then dried and extracted with acetone by percolation. The residues were extracted with 50% v/v alcohol by heating on boiling water bath under reflex for 3 h. The solvents were evaporated to have pasty mass, referred as hydroalcoholic extract of seed. The residues after alcoholic extraction were treated with 10% hydrochloric acid and boiled at 50°C for 2 h. The dry powdered roots were directly extracted with 50% v/v alcohol by heating on the boiling water bath under reflex for 4 h. The solvents were evaporated at room temperature to have pasty mass, referred as hydroalcoholic extract of root.

Chemicals and reagents: All the chemicals used were of analytical grade. Carrageenan and histamine were obtained from Otto and S.D. Fine chemicals Limited, Mumbai respectively. The solvents and reagents were also from S.D. Fine chemical Limited.

Pharmacological evaluation

Animals: Albino rats (Wistar strain) weighing 150-200 g of either sex were divided into different groups, each consisting of six animals. Animals were maintained on a commercial chew diet (Pranav Agro Industries Ltd., Sangli, Maharastra, India) and water *ad libitum* throughout the study period. This study was approved by the institutional animal ethics committee in accordance with the guidelines of Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA) (CPCSEA, 2003).

Drugs: All the extracts were suspended in distilled water using 1% w/v gum acacia. The reference drug indomethacine was dissolved in water. The control group received 1 mL of 1% w/v gum acacia in distilled water as vehicle. Pheniramine maleate, a reference standard was dissolved in the normal saline.

Lethal Dose, 50% (LD_{50}) determination: Animals were treated with different doses 250, 500, 750 and 1000 mg kg^{-1}, p.o. of each extracts. After single dose administration, animals were observed for death or any other deformities up to 72 h.

Preparation of ointment: CT seed and root extracts were suspended in distilled water using 1% w/v gum acacia in order to achieve a 25% w/v aqueous phase. The simple ointment base BP, as described in British Pharmacopoeia (1993), was melted by heating at 60°C over a water bath and the above aqueous phase was incorporated with constant stirring to yield an ointment containing 10% w/w extract. They were referred to as CT seed ointment and root ointment, respectively.

Wound healing activity

Excision wound model: The rats in these studies were inflicted with an excision wound as described by Morton and Malone (1972), under light ether anesthesia. Specifically, a single circular wound of ≈ 300 mm^2 was made on a depilated ethanol-sterilized dorsal thoracic region of the rats. The animals were then randomized into five groups (n = 6/group); each treatment outlined here was utilized each day (for 10 consecutive days) after the wound infliction. Rats in: Group 1 (control) received the earlier–described 2 mL vehicle (i.e., 1 mL of 1% gum acacia and 1 mL of 1% CMC solutions) only by gavage; Group 2 (ointment control) received a topical application of 50 mg of the Simple ointment BP; Group 3 (reference drug standard) were treated with a topical application of 50 mg of 1% (w/w) cotrimoxazole cream; Group 4 were treated with 500 mg CT seed extract/kg b.wt (by gavage); Group V were treated with 500 mg CT root extract/kg b.wt (by gavage); Group 4 were treated topically with 50 mg CT seed ointment and Group 5 were treated topically with 50 mg CT root ointment. For the determination of wound closure, the wound area was traced onto mm^2 graph paper on Days 0 and 10 and the percentage of wound closure was then calculated.

Incision wound model: In this model, 6 cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of rats as described by Ehrlich and Hunt (1969). The wounds were then closed with interrupted sutures 1 cm apart. The animals were then randomized into five groups (n = 6/group); each treatment outlined here was utilized each day (for 8 consecutive days) after the wound infliction. Rats in: Group 1 (control) received the earlier-described 2 mL vehicle (i.e., 1 mL of 1% gum acacia and 1 mL of 1% CMC solutions) only by gavage; Group 2 (ointment control) received a topical application of 50 mg of the BP ointment base; Group 3 (reference drug standard) were treated with a topical application of 50 mg of 1% (w/w) cotrimoxazole cream; Group 4 were treated with 500 mg CT seed extract/kg b.wt (by gavage); Group 5 were treated with 500 mg CT root extract/kg b.wt (by gavage); Group 6 were treated topically with 50 mg CT seed ointment and Group 7 were treated topically with 50 mg CT root ointment. The skin breaking strength of the wound was measured 2 day later, as described by Lee (1968).

Dead-space wound model: Dead-space wounds were created under light ether anesthesia by subcutaneous implantation of sterilized cylindrical grass piths (2.5×0.3 cm), one on either side of the dorsal paravertebral surface of the rat (Turner, 1965). Animals were then randomized into five groups (n = 6/group) each treatment outlined here was utilized each day (for 10 consecutive days) after the pith implantations. Rats in: Group 1 (control) received the earlier–described 2 mL vehicle (i.e., 1 mL of 1% gum acacia and 1 mL of 1% CMC solutions) only by gavage; Group 2 (ointment control) received a topical application of 50 mg of the simple ointment BP; Group 3 (reference drug standard) were treated with a topical application of 50 mg of 1% (w/w) cotrimoxazole ointment; Group 4 were treated with 500 mg CT seed extract/kg BW (by gavage); Group 5 were treated with 500 mg CT root extract/kg BW (by gavage); Group 6 were treated topically with 50 mg CT seed ointment and Group 7 were treated topically with 5b0 mg CT root ointment. To assess the extent of wound healing in the rats, the granulation tissues that had formed on the grass piths in each rat were excised on Day 10 post-wounding and weighed.

In each of the three wound healing activity studies performed here, rats were individually observed for 1.5 h after each topical application to make sure there was no medication spoilage. In addition, each animal group contained three extra rats (each treated with the given regimen, in parallel) in case there was any need to compensate for unexpected deaths or infections during the protocol periods. Any rat showing signs of infection during the study period was excluded from the study (and replaced by one of the alternates in its regimen).

Phytochemical analysis

Estimation of total phenolic compounds: The amount of total phenolics in the extracts was determined by the method of Rathee *et al*. (2006).

Estimation of total flavonoids: The amount of total Flavonoids in the extracts was determined by the reported method of Rathee *et al*. (2006).

High performance thin layer chromatography (HPTLC) fingerprinting: CT seed and root extracts were dissolved separately in alcohol to have 1 mg mL^{-1} solutions. 10 µL of solution was applied in duplicate on the TLC aluminum sheet pre-coated with silica gel 60 F254, having thickness of 0.2 mm (E merck, Germany) using CAMAG LINOMAT SAMPLER-V. The TLC was developed using mobile phase of ethyl acetate: Ethanol: Water (6:2:1) up to the distance of 7 cm. The TLC were scanned using CAMAG TLC SCANNER-III and photo-documented at 254 nm and 366 nm using CAMAG REPROSTAR-III.

Quantification of Kaempferol in CT seed extract by HPTLC: CT seed and root extracts, 1 g each were separately dissolved in 50 mL of methanol and mixed with

20 mL of 2N H_2SO_4. The mixtures were heated on boiling water bath under reflux for 4 h. The aqueous phase is than extracted with chloroform (5×50 mL). The chloroform fractions were combined, filtered and evaporated at room temperature to have pasty mass referred as acid hydrolyzed extracts of seed and root respectively. The acid hydrolyzed extracts were used for kaempferol quantification.

The acid hydrolyzed extracts of CT seed and root were dissolved in alcohol to have 1 mg mL^{-1} solutions. The standard solution of Kaempferol (1 mg mL^{-1}) is applied as 3, 6, 9, 12 and 15 µL on the TLC aluminium sheet precoated with silica gel 60 F254, having thickness of 0.2 mm (E merck, Germany) using CAMAG LINOMAT SAMPLER-V. 10 µL each of acid hydrolyzed extracts of CT seed and root were also applied. The TLC was developed using mobile phase benzene: diethyl ether: Hexane (8 : 6 : 2) up to the distance of 7 cm. The TLC were scanned using CAMAG TLC SCANNER-III and photo-documented at 254 nm and 366 nm using CAMAG REPROSTAR-III.

The standard curve was prepared by plotting area against ig of Kaempferol. The amount of Kaempferol in acid hydrolyzed CT extracts were calculated from the standard curve and expressed as (%) w/w of CT extracts.

Statistical analysis: Statistical analysis was carried out using one way ANOVA followed by Tukey's test, using the SigmaState™ 2.03 software and computer with Intel Pentium® dual core™ processor. A value of $p<0.05$ was considered a statistically significant difference between analyzed groups.

RESULTS

Since, CT seed and root showed significant anti-inflammatory and immunomodulatory activities, it is worthwhile to evaluate effect on wound healing activity. Therefore, in the present study we investigated wound healing activity of CT seed and root extracts using experimental animal models.

Excision wound model: In excision wound model, CT seed (97.67±0.19) and root (96.29±0.31) extracts significantly increased % wound healing when compared with the control group (72.74±0.66) and ointment control group (73.22±0.55). These effects were comparable with that of reference drug cotrimoxazole ointment (98.20±0.20). The topical application of CT seed and root extracts as ointment also significantly increased % wound healing (Table 1).

Table: 1 Effects of treatments on an excision wound model

	Wound area (mm^2)		
Group	Day 0	Day 10	%Wound healing
Control	301.17±3.54	71.50±1.43	72.74±0.66
Ointment control	303.16±3.00	69.00±2.28	73.22±0.55
Cotrimoxazole ointment	303.17±2.69	5.50±0.62	98.20±0.20*
CT seed extract	282.12±7.48	6.46±0.49	97.67±0.19*
CT root extract	367.12±8.56	13.67±1.20	96.29±0.31*
CT seed ointment	337.31±8.18	7.42±0.42	97.53±0.15*
CT root ointment	295.92±6.84	13.50±0.96	95.69±0.33*

All values represent Mean±SEM; n = 6 per treatment group. *: Significant when compared with the control and ointment control group. CT: *C. ternatea*

Table: 2: Effects of CT on an incision and dead space wound models

Groups	Tensile strength (g)	Wet granulation weight (g)
Control	284.75±8.10	0.41±0.02
Ointment control	297.02±9.59	0.43±0.01
Cotrimoxazole ointment	448.47±10.86*	0.86±0.01*
CT seed extract	552.58±8.10*	0.89±0.01*
CT root extract	568.52±11.74*	0.73±0.02*
CT seed ointment	489.08±8.11*	0.89±0.01*
CT root ointment	421.93±9.39*	0.73±0.02*

All the values were expressed as Mean±SEM, n = 6 per treatment group. *: Significant when compared with the control and the ointment control group. CT: *C. ternatea*

Incision wound model: In case of incision wound healing model, wound healing was measured as tensile strength. CT seed (552.58±8.10) and root (568.52±11.74) extracts produced significant increase in the tensile strength when compared with the control group (284.75±8.10) and the ointment control group (297.02±9.59) and effects were comparable with that of cotrimoxazole ointment (448.47±10.86). The topical application of both the extracts as ointment also produced similar effects (Table 2).

Dead space wound model: In case of dead space wound model, CT seed (0.86±0.01) and root (0.76±0.01) extracts significantly increased wet granulation weight as compared to control (0.41±0.02) and ointment control group (0.43±0.01) (Table 2). The topical application as ointment of hydroalcoholic extracts of seed (0.89±0.01) and root (0.73±0.02) produced significant increase in the wet granulation weight. The effects were comparable with the cotrimoxazole ointment group (0.86±0.01) (Fig. 1).

Total phenolic and Flavonoid contents of CT seed and root extracts: The total phenolic and flavonoid contents were measured in terms of gallic acid and catechin equivalents, respectively (Table 3).

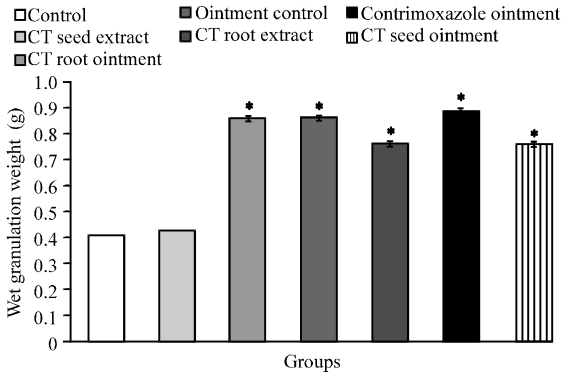

Fig. 1: Effects of CT on granulation weight in dead space wound model. CTSE: Acid hydrolyzed extract of *C. ternatea* seed extract and CTRE: Acid hydrolyzed extract of *C. ternatea* root extract

Table 3: The total phenolic and flavonoid contents of CT seed and root extracts

Plant extract	Phenolic content[a] (mg CA equivalents g^{-1})	Flavonoid content[a] (mg GA equivalents g^{-1})
CT seed extract	26.00±0.58	22.67±0.67
CT root extract	12.33±0.88	10.66±1.45

[a]All values are Mean±SEM (n = 3)

Table 4: Rf values and relative area of different bends of chromatogram of CT seed and root extracts developed using mobile phase Ethyl acetate: ethanol: water (6:2:1)

CT seed extract			CT root extract		
Peak	Rf	Area	Peak	Rf	Area
1	0.02	1943.0	1	0.02	3909.0
2	0.08	681.8	2	0.19	173.7
3	0.28	416.7	3	0.27	297.0
4	0.52	5556.7	4	0.39	1088.6
5	0.68	2049.4	5	0.47	1956.8
6	0.75	233.2	6	0.56	2041.8
7	0.81	3487.4	7	0.63	1129.8
8	0.76	3178.3	9	0.80	1786.9

CT: *C. ternatea*, Rf: Relative flow

TLC fingerprinting: The TLC fingerprinting of CT seed extract showed seven different bends (Fig. 2, 4) with Relative Flow (Rf) value of 0.02, 0.08, 0.28, 0.52, 0.68, 0.75 and 0.81. The bends with Rf values 0.02, 0.52, 0.68 and 0.81 were relatively major peaks as evident from the area of peaks (Table 4). The strongest peak is having Rf value of 0.52 and relative area of 5556.7.

The TLC fingerprinting of CT root extract showed 11 different bends (Fig. 2, 4), with Rf value of 0.02, 0.19, 0.27, 0.39, 0.47, 0.56, 0.63, 0.76 and 0.80. The bends with Rf values 0.02, 0.39, 0.47, 0.56, 0.63, 0.76 and 0.80 were relatively major peaks as evident from the area of peaks (Table 4). The strongest bend is having Rf value of 0.02 and relative area of 3909.0.

Quantification of Kaempferol in acid hydrolyzed extracts: The acid hydrolyzed extracts of CT seed and root extracts were used for the quantification of Kaempferol. Acid hydrolyzed extract of CT seed extract only showed the presence of Kaempferol (Fig. 5). The HPTLC was developed using Benzene: Diethyl ether: Hexane (4:3:1) mobile phase system. The standard curve of kaempferol is prepared (Table 5) and amount of kaempferol in acid hydrolyzed CT seed extract was determined. The amount of Kaempferol in acid hydrolyzed extract of CT seed extract was 0.06% w/w of CT seed extract.

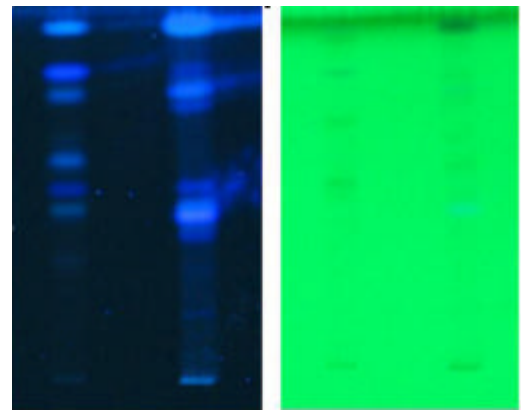

Fig. 2: Photograph of TLC of *C. ternatea* seed and root extracts developed using mobile phase of ethyl acetate: ethanol: water (6:2:1). CTSE: Acid hydrolyzed extract of *C. ternatea* seed extract and CTRE: Acid hydrolyzed extract of *C. ternatea* root extract

Table 5: Rf values and area of reference marker Kaempferol and acid hydrolyzed extract of CT seed extract

Sample	Amount (µg)	Rf	Area
K1	3	0.76	1529.5
K2	6	0.75	3248.1
K3	9	0.75	4806.3
K4	12	0.75	6361.1
K5	15	0.75	7869.6
CTSE	-	0.76	3254.1
CTRE	-	-	-

K: Kaempferol, CTSE: acid hydrolyzed extract of *C. ternatea* seed extract and CTRE: Acid hydrolyzed extract of *C. ternatea* root extract

DISCUSSION

Wound healing is a natural process of regenerating dermal and epidermal tissue. Whenever there is a wound, a set of overlapping events take place in a predictable fashion to repair the damage (Iba *et al*., 2004). The process has been conveniently categorized into phases such as the inflammatory, proliferative and remodeling

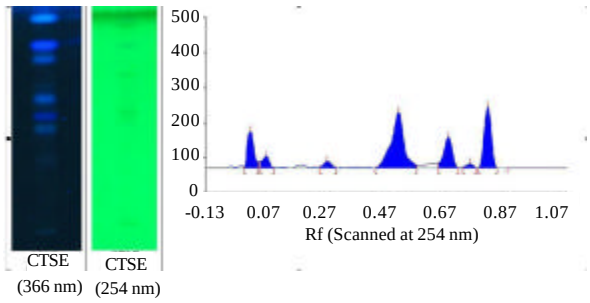

Fig. 3: Chromatogram of CT seed extract developed using mobile phase of ethyl acetate: ethanol: water (6:2:1)

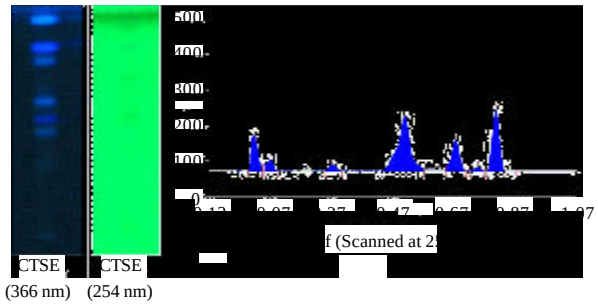

Fig. 4: Chromatogram of CT root extract developed using mobile phase of ethyl acetate: ethanol: water (6:2:1)

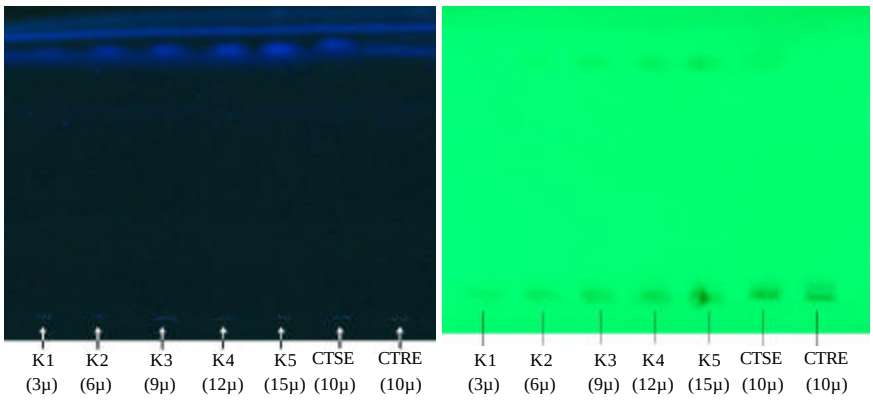

Fig. 5: Photograph of HPTLC of Kaempferol and acid hydrolyzed extract of CT seed and root extract developed using mobile phase system Benzene: Diethyl ether: Hexane (4:3:1) Photograph of HPTLC at 366 nm Photograph of HPTLC at 254 nm, K: kaempferol, ACSE: acid hydrolyzed extract of *C. ternatea* seed extract and ACTRE: Acid hydrolyzed extract of *C. ternatea* root extract

phases (Stadelmann *et al*., 1998). In the inflammatory phase, bacteria and debris are phagocytosed and removed and factors are released that cause the migration and division of cells involved in the proliferative phase. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction (Midwood *et al*., 2004). In epithelialization, epithelial cells crawl across the

wound bed to cover it (Chin *et al*., 2000). The wound is closed by a combination of all these and by the process of wound contraction. During wound contraction, the wound is made smaller by the action of myofibroblasts which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis. In the present study, CT seed and root extracts and its ointment in simple ointment base significantly decreased the wound area when compared with the control group. Increased wound contraction in CT seed and root extracts treated rats might be due to an enhanced activity of fibroblasts in regenerated wound tissues.

Myofibroblasts are believed to play a key role in wound contraction by exerting tension on surrounding extracellular matrix and secreting extracellular matrix protein such as collagen to stabilize the contraction (Suntar *et al*., 2009). Collagen is a major protein of extracellular matrix and component that ultimately contributes to wound strength (Singer and Clark, 1999). Hexosamine and uric acid are matrix molecules which act as ground substance for the synthesis of new extracellular matrix. There is a report of increase in the levels of these components during the early stage of wound healing followed by restoration of normal levels (Suguna *et al*., 2002). Therefore, the wound contraction and healing effects of CT could be attributed to increased synthesis of extracellular matrix proteins and ground substances. This is also supported by the increase in the tensile strength.

In incision wound model, increase in tensile strength of treated wounds may be due to an increase in collagen formation per unit area and stabilization of the fibers (Mukherjee *et al*., 2000). The tensile strength depends upon the Van der Waals force interaction among the hydrogen ion bonds of the triple helix collagen, leading to twisting of the collagen fibers. The more twisting of these fibers that occurs, the greater the tensile strength and hence the better the healing of wounds (Mian *et al*., 1992). Deposition of newly synthesized collagen at the wound site increases the collagen concentration per unit area and hence, the tissue tensile strength (Suguna *et al*., 2002). In the present study, CT significantly increased the tensile strength of the wound. This could therefore be attributed to increased synthesis and deposition of collagen.

In dead space wound model, granulation tissue formation is indicative of proliferative and remodeling phase of wound healing process. The granulation tissue of the wound is primarily composed of edema, fibroblasts, collagen and new blood vessels. The mesenchymal cells of the wound area adjust themselves into fibroblasts then begin migrating into the wound gap together with the fibrin strands (Pesin *et al*., 2009). In the present study, CT seed and root significantly increased the granulation weight, indicating that there might be increased protein synthesis and improvement of both proliferative and remodeling phases of the wound healing.

Thus, in the present study, CT showed profound wound healing activity against various experimental wound models, affecting all the phases-wound contraction, proliferative and remodeling-of wound healing. The free radicals and oxidative reaction products produce tissue damage and play a major role in the aggravation of tissue damage during wound healing (Sonel *et al*., 1997). Several antioxidants like curcumin and vitamin E have been reported to quench oxidative damage to the tissue (Taczolowski *et al*., 1992; Sieradzki *et al*., 1998). Since, CT showed significant anti-oxidant and anti-inflammatory activities in our previous studies, the wound healing activity could be attributed to these properties. Similar types of wound healing effects have also been reported in different plants (Manjunatha *et al*., 2005; Nayak *et al*., 2009). Further, the flavonoids are reported to have therapeutic uses due to their anti-inflammatory, anti-fungal, anti-oxidant and wound healing properties (Okuda, 2005; Nayak *et al*., 2009). Flavonoids are also known to endorse the wound healing process primarily due to their anti-microbial and astringent properties which appears to be responsible for wound contraction and elevated rate of epithelialization (Tsuchiya *et al*., 1996). CT is also reported to contain flavonoids (Kulshrestha and Khare, 1968) and have antimicrobial activity (Kelemu *et al*., 2004). Hence, the wound healing activity of CT could be attributed to the presence of flavonoids and phenolic compounds.

CONCLUSION

The present experimental study demonstrated wound healing properties of *C. ternatea* root and seed extracts. The anti-inflammatory and immunomodulatory actions of plant extracts can be attributed to wound healing activity. The presence of flavonol glycosides and phenolic compounds may be responsible for this activity. Further exploration of mechanism of wound healing activity may contribute to the knowledge.

ACKNOWLEDGMENT

Authors are thankful to Gujarat Council for Science and Technology for their financial assistance.

REFERENCES

Anonymous, 2003. The Ayurvedic Pharmacopoeia of India. Department of Indian system of medicine and homoeopathy, Ministry of health and family welfare, Government of India, New Delhi.

British Pharmacopoeia, 1993. The British Pharmacopoeia. Vol. 1, HMSO Publication Center, London, pp: 604-605.

CPCSEA, 2003. CPCSEA guidelines for laboratory animal facility. Indian J. Pharmacol., 35: 257-274.

Chin, G.S., E.J. Stelnicki, G.K. Gittes and M.T. Longaker, 2000. Characteristics of Fetal Wound Repair. In: Scarless Wound Healing, Garg, H.G. and M.T. Longaker (Eds.). Marcel Dekker, New York, pp: 239-257.

Chopra, R., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. 1st Edn., National Institute of Science Communications, New Delhi, India.

Dovi, J.V., A.M. Szpaderska and L.A. DiPietro, 2004. Neutrophil function in the healing wound: Adding insult to injury. Thromb. Haemost., 92: 275-280.

Ehrlich, H.P. and T.K. Hunt, 1969. Effect of cortisone and anabolic steroids on tensile strength of a healing wounds. Ann. Surg., 170: 203-206.

Eming, S.A., T. Krieg and J.M. Davidson, 2007. Inflammation in wound repair: Molecular and cellular mechanisms. J. Invest. Dermatol., 127: 514-525.

Gurtner, C.G., S. Werner, Y. Barrandon and M.T. Longaker, 2008. Wound repair and regeneration. Nature, 453: 314-321.

Iba, Y., A. Shibata, M. Kato and T. Masukawa, 2004. Possible involvement of mast cells in collagen remodeling in the late phase of cutaneous wound healing in mice. Int. Immunopharmacol., 4: 1873-1880.

Kelemu, S., C. Cardona and G. Segura, 2004. Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea*, a tropical forage legume. Plant Physiol. Biochem., 42: 867-873.

Kirtikar, K.R. and B.D. Basu, 1976. Indian Medicinal Plants. Periodical expert book agency, Delhi.

Kulshrestha, D.K. and M.P. Khare, 1968. Chemical study of *Clitorea ternatea* seeds. Chem Ber., 101: 2096-2105.

Lee, K.H., 1968. Studies on mechanism of action of salicylates II. Retardation of wound J. Pharm. Sci., 57: 1042-1043.

Malabadi, R.B., G.S. Mulgund and K. Nataraja, 2005. Screening of antibacterial activity in the extracts of *Clitoria ternatea*. J. Med. Arom. Plant Sci., 27: 26-29.

Manjunatha, B.K., S.M. Vidya, K.V. Rashmi, K.L. Mankani, H.J. Shilpa, S. Singh and D. Jagadeesh, 2005. Evaluation of wound-healing potency of *Vernonia arborea*. Ind. J. Pharmacol., 37: 223-226.

Martin, P., D. D'Souza, J. Martin, R. Grose, L. Cooper, R. Maki and S.R. McKercher, 2003. Wound healing in the PU.1 null mouse: Tissue repair is not dependent on inflammatory cells. Curr. Biol., 13: 1122-1128.

Martin, P. and S. Leibovich, 2005. Inflammatory cells during wound repair: The good, the bad and the ugly. Trends Cell Biol., 15: 599-607.

Mian, M., F. Beghe and E. Mian, 1992. Collagen as a pharmacological approach in wound healing. Int. J. Tissue React., 14: 11-14.

Midwood, K.S., L.V. Williams and J.E. Schwarzbauer, 2004. Tissue repair and the dynamics of the extracellular matrix. Int. J. Biochem. Cell Biol., 36: 1031-1037.

Morton, J.J.P. and M.H. Malone, 1972. Evaluation of vulnerary activity by an open wound procedure in rats. Arch. Int. Pharmacodyn. Ther., 196: 117-126.

Mukherjee, P.K., R. Verpoorte and B. Suresh, 2000. Evaluation of *in vivo* wound healing of *Hypericum patulum* leaf extract on different wound models in rats. J. Ethanopharmacol., 70: 315-321.

Mukherjee, P.K., V. Kumar, N.S. Kumar and M. Heinrich, 2008. The ayurvedic medicine *Clitoria ternatea*-from traditional use to scientific assessment. J. Ethanopharmacol., 120: 291-301.

Nayak, B.S., S. Sandiford and A. Maxwell, 2009. Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia* L. leaf. Evid.-Based Comp. Alternat. Med., 6: 351-356.

Okuda, T., 2005. Systematic and health effects of chemically distinct tannins in medicinal plants. Phytochem., 66: 2012-2031.

Pesin, I., U. Koca, H. Keles and E. Kupeli Akkol, 2009. Wound healing activity of *Rubus sanctus* schreber (Rosaceae): Preclinical study in animal models. ECAM, 2009: 1-7.

Rajan, V. and R.Z. Murray, 2008. The duplicitous nature of inflammation in wound repair. Wound Practice Res., 16: 122-129.

Rathee, J.S., S.A. Hassarajani and S. Chattopadhyay, 2006. Antioxidant activity of *Mammea longifolia* bud extracts. Food Chem., 99: 436-443.

Schrementi, M.E., A.M. Ferreira, C. Zender and L.A. DiPietro, 2008. Site-specific production of TGF-beta in oral mucosal and cutaneous wounds. Wound Repair Regen., 16: 80-86.

Sieradzki, E., E. Olejarz, K. Strauss, A. Marzec, M. Mieszkowska and J. Kauzringny, 1998. The effect of selenium and vitamin E on the healing process of experimental corneal lesions in eye of the rabbit. Klin. Oczna., 100: 85-88.

Singer, A.J. and R.A.F. Clark, 1999. Cutaneous wound healing. N. Engl. J. Med., 341: 738-746.

Solanki, Y.B. and S.M. Jain, 2010a. Anti-hyperlipidemic activity of *Clitoria ternatea* and *Vigna mungo* in rats. Pharmaceut. Biol., 48: 915-923.

Solanki, Y.B. and S.M. Jain, 2010b. Immunomodulatory activity of Ayurvedic plant Aparajita (*Clitoria ternatea* L.) in male albino rats. Global J. Sci. Front. Res., 10: 2-8.

Solanki, Y.B. and S.M. Jain, 2011. Hepatoprotective effects of *Clitoria ternatea* and *Vigna mungo* against acetaminophen and carbon tetrachloride-induced hepatotoxicity in rats. J. Pharmacol. Toxicol., 60: 30-48.

Sonel, O., O. Cetinkale, G. Ozbay, F. Ahcloglu and R. Bulan, 1997. Oxygen free radicals impair wound healing in ischemic rat skin. Ann. Plast. Surg., 39: 516-523.

Stadelmann, W.K., A.G. Digenis and G.R. Tobin, 1998. Physiology and healing dynamics of chronic cutaneous wounds. Am. J. Surg., 176: 26S-38S.

Stramer, B.M., R. Mori and P. Martin, 2007. The inflammation-fibrosis link: A Jekyll and J. Invest. Dermatol., 127: 1009-1017.

Suguna, L., S. Singh, P. Sivakumar, P. Sampath and G. Chandrakasan, 2002. Influence of *Terminalia chebula* on dermal wound healing in rats. Phytother. Res., 16: 227-231.

Suntar, I.P., U. Koca, K.A. Esra, D. Yýlmazer and M. Alper, 2009. Assessment of Wound Healing Activity of the Aqueous Extracts of *Colutea cilicica* Boiss. and Bal. Fruits and Leaves. ECAM, 2011: 1-7.

Taczolowski, J., T. Wolski and K. Klamut-Sory, 1992. Effect of drugs inhibiting the formation of hydroxide radicals on healing of experimental corneal ulcers. Klin. Oczna., 94: 83-85.

Tsuchiya, H., M. Sato, T. Miyazaki, S. Fujiwara and S. Tanigaki *et al*., 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin resistant *Staphylococcus aureus*. J. Ethnopharmacol., 50: 27-34.

Turner, R.A., 1965. Screening Methods in Pharmacology. Academic Press, New York..

Upadhye, A.S. and M.S. Kumbhojkar, 1993. Studies on the Ayurvedic drug shankhapushpi from western maharashtra: Medico-botanical aspect. Bull. Medico-Ethano Bot. Res., 14: 64-69.

Yadava, R.N. and V. Verma, 2003. Antimicrobial activity of a novel flavonol glycoside Asian J. Chem., 15: 842-846.