Research Article

Potential Wound Healing Activity of *Euphorbia Hirta* Linn Total Flavonoid Fraction

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Accepted on: 23-07-2013; **Finalized on:** 30-09-2013.

ABSTRACT

Euphorbia hirta (Euphorbiaceae) has reported antimicrobial, antifungal, antiviral anti inflammatory, anti arthritic and antioxidant effect with presence of polyphenolic and flavonoid compounds lead us to evaluate the wound healing activity of enriched flavonoid fraction. Qualitative phytochemical test and quantitative estimation of total flavonoid and polyphenol content was carried out on ethanol, methanol and water extract of *E. hirta* whole plant followed by separation of total flavonoid fraction (EHTF) from methanol extract. TLC analysis showed presence of quercetin, ferulic acid and gallic acid in total flavonoid fraction of *E. hirta* whole plant. Wound healing activity of EHTF was determined on incision, excision and dead space wound model on rat at 200, 400 and 600 mg/kg, p.o following acute toxicity study. Wound healing was assessed by rate of wound contraction, epithelisation period, tensile strength and estimation of granulation tissue weight, hydroxyproline, SOD, catalase and total protein content. EHTF (600 mg/kg) significantly increased the wound breaking strength, granulation tissue weight and hydroxyproline content ($p < 0.001$). Antioxidant enzyme SOD and catalase level was found to be higher in wound tissue along with total protein content ($p < 0.01$). Epithelialize period was faster and the rate of wound contraction was significantly higher ($p < 0.05$-0.001) in EHTF 400 and 600 mg/kg treated groups. Increased tensile strength, wound contraction rate and hydroxyproline content provide strong evidence for would heal property of *E. hirta* flavonoid. Presence of myricitrin, quercitrin, kaempferol, luteolin and gallic acid like polyphenolic compounds in *E. hirta* indicate potential scavenging effect as important determinant of wound healing property. Anti inflammatory, reduced macrophage and granulocyte infiltration, reduced cartilage degeneration and antimicrobial effect may also have contributed towards wound healing.

Keywords: Antioxidant, *Euphorbia hirta,* Flavonoid, Hydroxyproline, Wound healing.

INTRODUCTION

From prehistoric times, various communities and civilizations throughout the world are using herbal medicines. For the past several decades, people are increasingly consuming herbal medicines without prescription. They are traditionally considered as harmless since they belong to natural sources. Herbal formulations have reached widespread acceptability as therapeutic agents like anti diabetics, anti arthritics, aphrodisiacs, hepatoprotective, cough remedies, memory enhancers and adaptogens. India has a rich tradition of plant-based extracts/decoction/pastes used by tribal and folklore practitioner for treatment of cuts, wounds, and burns.[1] Wounds are physical injuries that result in an opening or break of the skin that causes disturbance in the normal skin anatomy and function. The restoration of integrity to injured tissues takes place by replacement of dead tissue with viable tissue. It is an intricate process in which the skin repairs itself after injury.[2]

Euphorbia hirta Linn. (Euphorbiaceae) was selected for the present study considering its use in traditional medicine after thorough literature search to find out scientific basis of the claimed therapeutic potentials. *E. hirta* is an extensively studied plant by the scientific community. The crude extract of plant has been reported for antimicrobial[3-5], antifungal[5] and antiviral[6] effect by number of researchers. The antimicrobial property is well established against wide range of bacteria. The plant is also reported to have antiinflammatory[7,8], antiarthritic[9], antioxidant[10-12] and antianaphylactic[13] activity.

All these properties along with rich presence of flavonoid and polyphenols in alcoholic and aqueous extracts has lead us conceive the present study regarding exploration of wound healing efficacy. Flavonoid content by virtue of their antimicrobial, antioxidant and anti inflammatory property is expected to exert potential wound healing effect which till now has not been explored, so the present study aims at evaluation of wound healing efficacy of flavonoid rich fractions of *E. hirta* whole plant along with evaluation of *in-vivo* antioxidant activity.

MATERIALS AND METHODS

Plant material

The whole plant of *E. hirta* was collected from the field of Bhopal, India in the month of Jan–Feb., 2011. The plant was authenticated by botanist, Dr. (Mrs.) Madhuri Modak, Professor, Dept. of Botany, Govt. Motilal Vigyan Mahavidyalaya, Bhopal. A voucher specimen no: MVM-h-H/Am-208 was assigned and kept for future references.

Preparation of extract

The whole plant was completely dried under shade for 5-6 days, crushed coarsely and stored in well closed container. The extraction of whole plant was performed by cold maceration with 3 different solvents viz. ethanol, methanol and double distilled water for 72 hours. The

solvents were distilled off and the resulting semisolid mass was dried and yield calculated for ethanol (EE), methanol (ME) and water extract (WE) separately.

Phytochemical analysis

Qualitative tests

Various qualitative test were carried out for presence of flavonoids (Shinoda test, alkaline reagent test and zinc hydrochloride test), alkaloid (Dragendorff's reagent, Hager's reagent, Mayer's reagent, Wagner's reagent), glycoside, saponin (froth test), tannin (ferric chloride test), steroid and triterpenoid (Liebermann-burchard's, Salkowski's test) in extracts.[14]

Estimation of total flavonoids

Aluminium chloride technique was used for flavonoid estimation and flavonoid content was expressed as percentage of quercetin equivalent per 100 gm dry weight of sample.[15] Aluminium chloride colorimetric technique was used for flavonoids estimation. EE, ME and WE respectively 0.5 ml (1 gm/10 ml) in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 minute after which the absorbance of the reaction mixture was measured at 415 nm with a double beam UV spectrophotometer (EI model no. 1372, Japan). The calibration curve was plotted by preparing the quercetin solutions at concentrations 10 to 100 µg/ml in methanol. Amount of flavonoid was calculated by the equation of standard quercetin graph.

Estimation of total polyphenol

The total phenolic contents of EE, ME and WE were determined using the Folin-Ciocalteu assay.[16] This colorimetric method is based on the reduction of a phosphotunstate-phosphomolybdate complex by phenolics to blue reaction products in alkaline conditions. EE, ME and WE respectively 0.5 ml (1 gm/10 ml) added with 250 µl of Folin-Ciocaltue reagent and 1.25 ml of 20% aqueous sodium carbonate solution. After incubation of 45 minute in dark, the absorbance was spectrophotometrically recorded at 725 nm against the blank (0.5 ml of extract was replaced by 0.5 ml of extracting solvent). TPC was determined in comparison with standard gallic acid equivalent (mg/gm of dry mass), which is a common reference compound.

Enrichment of flavonoid in methanolic extract

Methanolic extract was subjected to separation and enrichment of total flavonoid on the basis of quantitative estimation. ME was subjected to alkaline hydrolysis with 2M NaOH and boiled for 30 minutes. The mixture was cooled and neutralised with 2M HCl and aqueous phase was extracted with n-butanol. Finally n-butanol phase was separated, dried (yield 7.66%) and stored for further study designated as *E. hirta* total flavonoid (EHTF) fraction.[17]

Thin layer chromatography

Freshly coated plates with silica gel G_{254} was allowed to air dry in room temperature and transferred to oven for activation maintained at 110°C for 30 min. EHTF was dissolved in methanol and filter through Whatman filter paper. Solvent system described in literature for flavonoids toluene, ethyl acetate and formic acid in the ratio of 5: 3:2 was attempted to achieve better resolution.[18] The plates were placed into the developing chamber and allowed to run until it reaches a height of about 10 cm from the point of spotting. Spraying agent used for detection of spots was vanillin sulphuric acid reagent. After development the plates were kept in oven maintained at 110°C to optimal color development. Standard samples of quercetin (JPN Pharma, Mumbai) and Gallic acid (Phytomed Pharma, Rajkot) was gift samples, and ferulic acid was purchased from Himedia (Mumbai), used for co-TLC to compare the Rf values.

Experimental animals

Male Wistar rats (*Rattus norvegicus*) with a mean weight of 175 ± 10 gm were kept in clean polypropylene cages placed in a well-ventilated animal house (temperature 23 ± 1°C, photoperiod 12 hrs light and dark cycle each throughout the experimental period; humidity 45 - 50%). The rats were allowed free access to water *ad libitum* and feed with standard commercial pelleted chaw (Hindustan liver, India). The experiment was carried out following approval by the Institutional Animal Ethical Committee of Radharaman College of Pharmacy, Bhopal (M.P.), registered under CPCSEA, India (IAEC approval no. IAEC/RCP/March-2012/08) and were in accordance with the guidelines of the CPCSEA, Chennai, India.

Acute toxicity study (LD_{50})

EHTF was suspended in 2% aqueous carboxy methyl cellulose for per oral administration. LD_{50} was determined according to the guidelines of Organization for Economic Co-operation and Development (OECD) following the up and down method (OECD guideline No. 423) and fixed dose method (OECD guideline No. 420). Based on these guidelines a limit test was performed at 2000 mg/ kg, p.o. to categorize the toxicity class (LD_{50}) of EHTF. A dose range of 200, 400 and 600 mg/kg was selected for the pharmacological activity study of EHTF as it was free of acute oral toxicity.[19]

Wound healing activity

Animals were divided into five groups of six rats in each as follows:

Group I : Vehicle control (0.5 ml/100 gm, p.o.)

Group II : Standard drug treated

Group III: EHTF (200 mg/kg, p.o.)

Group IV: EHTF (400 mg/kg, p.o.)

Group V: EHTF (600 mg/kg, p.o.)

Animals of group II was treated with dexamethasone (0.34, i.m) for incision and dead space model and with topical povidone iodine for excision wound model.

Incision wound model

All the animals were treated with vehicle, dexamethasone and different doses of EHTF daily for 7 days following wounding. Two 6 cm long para-vertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat. Wounds were closed with interrupted sutures 1 cm apart. The sutures were removed on the 7th post wounding day 2 hours after drug treatment. Wound-breaking strength was measured in anesthetized rats on the tenth day by continuous water flow technique of Lee.[20]

Dead space wound model

All the animals were treated with vehicle, dexamethasone and different doses of EHTF daily for 10 days following wounding. Dead space wounds were created through a small transverse incision made in the lumbar region. A polypropylene tube (2.5×0.5 cm) was implanted subcutaneously beneath the dorsal paravertebral lumbar skin. The day of the wound creation was considered as day zero.[21] On the 10th day 2 hours after drug treatment, the harvested granulation tissues were carefully dissected out along with the tubes. The tubular granulations were cut along their lengths and the pieces of granulation tissues were collected, dried at 60°C overnight and weighed. The granulation mass was placed in sealed tubes containing 10 ml of 6 N HCl. The sealed tubes were heated at 110°C for 24 hrs to hydrolyse these tissues. The hydrolysate was cooled and excess of acid was neutralized with 10 N NaOH using methyl red as indicator. The volume of neutral hydrolysate was made up to 20 ml with distilled water. This was used to estimate hydroxyproline[22], catalase[23], superoxide dismutase[24] and protein[25] content.

Excision wound model

All the animals were treated with vehicle, dexamethasone and different doses of EHTF daily for 21 days following wounding. An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear using a round seal of 2.5 cm diameter ether anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2 diameter. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline.[26] The wound areas was determined by tracing wound on 1 mm^2 graph paper on the day of wounding and subsequently on every 4th day until healing were completed. Changes in the wound area were calculated, giving an indication of the percentage of wound contraction using following formula. The number of days require for falling of the scar without any residual raw wound was determined as the period of epithelialisation.[22]

$$\% \text{ Wound contraction} = \frac{\text{Initial wound size - Specific day wound size}}{\text{Initial wound size}}$$

Statistical analysis

The results are expressed as Mean ± SEM. The statistical comparison was performed using one way analysis of variance (ANOVA) to assess the statistical significance, followed by Tukey-Kramer Multiple Comparisons Test. P value less than 0.05 were considered statistically significant. Graph Pad Prism Version 3.02 was used for statistical analysis.

RESULTS

Phytochemical analysis

Yield of ethanol, methanol and water extract was 9.93%, 9.14% and 20.49% w/w respectively. Qualitative tests showed that all three extracts contain various types of phytochemicals viz. alkaloids, flavonoids, glycosides, amino acids, tannins, saponins and protein.

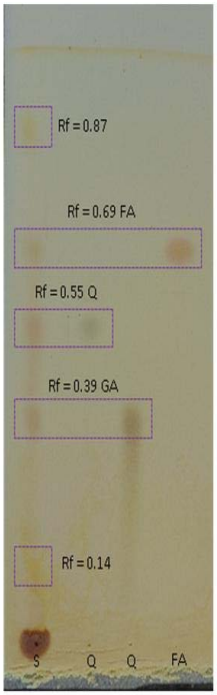

Figure 1: TLC analysis of *E. hirta* total flavonoid (EHTF) fraction showing flavonoid profile. S: EHTF (Rf 0.14, 0.39, 0.55, 0.69 and 0.87); Q: Quercetin (Rf 0.55); GA: Gallic acid (0.39) and FA: Ferulic acid (Rf 0.69).

Quantitative estimation for total flavonoids on ME, EE and WE resulted to be respectively 212.6, 149.8 and 146.5 mg/gm. Total polyphenol content was 76.75, 85.75 and 55.38 mg/gm in ME, EE and WE. Quantitative estimation showed relatively higher total flavonoid and polyphenol content in ME among the three solvents, which was processed further for separation of total flavonoid fraction, EHTF yielded to be 6.24%.

TLC analysis

EHTF showed presence of three flavonoidal compounds in matching Rf test as quercetin, gallic acid and ferulic acid with two unidentified compounds (Figure 1)

Acute toxicity study

OECD based Limit test showed that EHTF was free of acute oral toxicity upto 2000 mg/ kg, p.o. dose.

Incision wound healing

The EHTF 200 mg/kg treated group did not alter the breaking strength as compare to control while, 400 and 600 mg/kg extract treated group a significantly ($p < 0.05$-0.001) increased tensile strength comparable to dexamethasone (Table 1).

Dead space wound healing

In dead space wound model the dry weight of granulation tissue was 7.69 ± 0.68, 13.09 ± 0.33 and 15.55 ± 1.27 respectively in vehicle control, EHTF 600 mg/kg and dexamethasone treated group. In granulation tissue, hydroxyproline content of 600 mg/kg EHTF and standard treated group has increased extreme significantly ($p < 0.001$) compared to control (Table 1). SOD, catalase and total protein level in the granulation tissue was significantly ($p < 0.05$-0.01) increased in 400 and 600 mg/kg EHTF treated group compared to control (Table 2).

Table 1: Effect of *E. hirta* total flavonoid (EHTF) fraction on incision and dead space wound healing potential of rat

Treatment (mg/kg, p.o)	Incision wound breaking strength (gm)	Dead space wound model	
		Dry weight of granulation tissue (mg/100 gm of rat)	Hydroxyproline content (mg/gm of tissue)
Vehicle control	279.04 ± 12.41	7.69 ± 0.68	16.09 ± 1.35
Dexamethasone (0.34, i.m)	491.01 ± 13.90***	15.55 ± 1.27***	28.45 ± 2.30***
EHTF (200)	277.73 ± 10.31ns	8.83 ± 0.43*	15.89 ± 1.28ns
EHTF (400)	286.90 ± 11.94*	9.45 ± 0.61**	17.89 ± 2.26**
EHTF (600)	396.19 ± 18.60***	13.09 ± 2.33***	24.14 ± 2.23***

n = 6; values are in *Mean* ± *SEM*, data analysed by Tukey-Kramer Multiple Comparisons Test; ***p < 0.001; **p < 0.01; *p < 0.05; ns = non significant.

Table 2: Effect of *E. hirta* total flavonoid (EHTF) fraction on biochemical parameters of dead space wound healing potential on rat

Treatment (mg/kg, p.o)	Catalase activity (moles H_2O_2 consumed/min./mg protein)	Superoxide dismutase (units/mg of protein)	Total protein (mg/ 100 mg of granulation tissue)
Vehicle control	0.46 ± 0.02	1.15 ± 0.12	2.60 ± 0.06
Dexamethasone (0.34, i.m)	0.88 ± 0.04**	6.0 ± 0.12**	5.06 ± 0.48**
EHTF (200)	0.45 ± 0.03ns	1.16 ± 0.06ns	2.69 ± 0.07ns
EHTF (400)	0.52 ± 0.09*	2.63 ± 0.15**	3.34 ± 0.05**
EHTF (600)	0.75 ± 0.19**	5.06 ± 0.09**	4.02 ± 0.03**

Table 3: Effect of *E. hirta* total flavonoid (EHTF) fraction on excision wound healing potential of rat

Treatment (mg/kg, p.o)	Wound Area (mm^2)						Period of epithelization (days)
	0th day	4th day	8th day	12th day	16th day	20st day	
Vehicle control	299.54 ± 11.29	269.98 ± 10.22	220.51 ± 10.23	136.01 ± 9.16	71.65 ± 3.21	9.05 ± 0.82	21.50 ± 1.22
Povidone iodine (Topical)	300.28 ± 10.21	254.56 ± 9.55***	204.36 ± 9.29***	108.65 ± 8.11***	10.67 ± 0.94***	--	17.33 ± 1.28***
EHTF (200)	299.83 ± 12.36	269.00 ± 12.36ns	220.50 ± 8.11ns	135.99 ± 7.29ns	71.01 ± 4.25ns	--	19.66 ± 2.85ns
EHTF (400)	300.26 ± 13.45	267.56 ± 11.45**	219.56 ± 9.10*	134.55 ± 8.29**	69.98 ± 3.34**	--	19.50 ± 2.63*
EHTF (600)	298.82 ± 10.49	261.89 ± 10.50***	210.43 ± 10.22***	115.65 ± 9.21***	6.02 ± 0.79***	--	17.50 ± 1.56***

n=6; values are in *Mean* ± *SEM*, data analysed by Tukey-Kramer Multiple Comparisons Test; ***p < 0.001; **p < 0.01; *p < 0.05; ns = non significant.

Table 4: Effect of *E. hirta* total flavonoid (EHTF) fraction on percent wound contraction in excision wound of rat

Treatment (mg/kg, p.o)	Wound contraction (%)					
	0th day	4th day	8th day	12th day	16th day	20st day
Vehicle control	0	12.86	30.38	58.59	80.07	96.07
Povidone iodine (Topical)	0	18.22	35.94	83.81	97.04	100
EHTF (200)	0	13.28	31.45	74.64	90.31	100
EHTF (400)	0	14.89	33.87	75.18	94.69	100
EHTF (600)	0	17.35	38.57	81.29	98.08	100

Excision wound healing

In excision wound model, EHTF 400 and 600 mg/kg treated animals showed a significant ($p < 0.05$-0.001) reduction in the wound area and epithelization period compared to control (Table 3). The period of complete epithelialisation was reduced to 17.50 ± 1.56 day in 600 mg/kg treated group compared to 21.50 ± 1.22 days of control, whereas povidone iodine has taken 17.33 ± 1.28 days. The wound area closure was 80.07%, 97.04%, 94.69% and 98.08% respectively for vehicle, povidone iodine and EHTF at 400 and 600 mg/kg dose on 16th day (Table 4).

DISCUSSION

In normal skin the outermost layer epidermis and inner layer dermis forms a protective barrier. Due to injury once the protective barrier is broken a set of complex biochemical events takes place orchestrating the cascade of damage repair.[27] The wound healing process is divided into sequential, yet overlapping phases like haemostasis, inflammatory proliferation and remodelling.[28] Wound healing restores cellular structures and tissue layers in damaged tissue near to its normal state which dependent upon the type and extent of damage and the general state host's health ability of tissue repair. Wound contraction occurs throughout the process of healing, involving fibroblastic tissues to assist wound shrinkage. Different process such as granulation, collagenation, collagen maturation and scar formation occur concurrent but independently in the healing duration.[29]

Qualitative and quantitative estimation of phytochemicals were done for all the three solvents viz. methanol, ethanol and aqueous extracts. The preliminary phytochemical analysis of the plant extract showed the presence of tannins, triterpenoids, flavonoids and alkaloids. All these phytochemical constituents present in *E. hirta* can contribute to the wound healing activity. Recent studies have shown that phytochemical constituents like flavonoids and tannins are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialisation.[30] Quantitative estimation showed higher content of total flavonoids in methanolic extract and total polyphenol was maximum in ethanolic extract but with moderate quantity of flavonoids. Methanolic extracts was chosen to separate out total flavonoids which showed presence of quercetin, gallic acid and ferulic acid along with two other unidentified compounds. The LD_{50} of EHTF was found to be more than 2000 mg/kg, p.o. in acute toxicity testing. The under test doses 200,400 and 600 mg/kg was calculated as per 1/10th, 1/20th and 1/30th of the lethal dose respectively for the purpose of wound healing investigation. The effect of EHTF was screened on incision, dead space and excision wound model concurrently in reference to standard drug dexamethasone and topical povidone iodine.

Flavonoid extract of *E. hirta* showed a significant increase skin tensile strength along with increase in granulation tissue weight, protein and hydroxyproline content in incision and dead space wounded animals. Wound healing starts with an acute inflammatory phase followed by epithelization, angiogenesis and collagen deposition in the proliferative phase. Tissue repair after injury depends on the synthesis of a fibrous extracellular matrix to replace damaged tissue, which is then re-modelled over time to emulate normal tissue. Acute inflammation, re-epithelialisation, and contraction all depend on cell-extracellular matrix interactions this minimize infection and promote rapid wound closure.[31] Granulation tissue formed in the proliferative phase is primarily composed of fibroblasts, collagen and newly formed blood vessels.[32] The increased dry granulation tissue weight in the EHTF treated animals suggest higher fibroblast proliferation and collagen deposition which was ascertained with increased protein and hydroxyproline content found in wound tissue.

The importance of collagen in wound healing is highly appreciated because of the simple reason that the ultimate outcome of tissue repair in the higher vertebrate is the formation of scar tissue composed of collagenous fibres.[33] Collagen composed of the amino acid and hydroxyproline strengthen and support extra cellular tissue and hydroxyproline is used as a biochemical marker for tissue collagen turnover rate estimation.[34] The observed increase in collagen, an important constituent of extracellular matrix in the treated animals confirmed the positive effects of EHTF toward cellular proliferation, granulation tissue formation and epithelisation. The increased protein content is predominantly due to enhanced collagen synthesis in the EHTF treated groups.

E. hirta showed decrease in the epithelisation period, as evidenced by the shorter period for the fall of eschar

tissue in turn facilitating the rate of wound contraction. Wound contracture occurs throughout the healing process resulting in shrinkage of wound area. EHTF accelerates wound contraction may due to increased number of myofibroblast recruited into the wound area enhancing the contractile property. EHTF (600 mg/kg) showed significant increase the wound contraction where nearly 98% of wound area was contracted on 16th day. Hence it appears that higher dose of EHTF was able to promote epithelisation either by facilitating proliferation or increasing the viability of epithelial cells.[35]

Studies on the estimation of antioxidant enzyme level in the wound tissue revealed that the EHTF has significantly increased superoxide dismutase and catalase, the two powerful antioxidant enzymes known to quench and neutralize superoxide radicals. These antioxidant enzymes prevent cell damage caused by free radicals accelerating the healing process.[36] Lipid peroxidation is an important process in several types of injuries like burns, infected wounds and skin ulcers. Drug and herbal extracts that inhibits lipid peroxidation preventing cell damage, improve circulation and also promote DNA synthesis by way can increase strength of collagen fibres.[34]

Flavonoids are a large group of natural product widely present in fruits, vegetable, chocolates, herbs and beverages such as wine, tea or beer. Flavonoids are reported to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important determinants of wound healing. Bioflavonoids are thought to benefit connective tissue turnover by biding to elastin, preventing its degradation by elastases. The flavonoids and saponins present in this herb can speed up wound healing by strengthening the connective tissues.[37] Extensive research has been done on the role of flavonoid as antioxidants and also its relation with wound healing.[38-40] Flavonoids are reported to reduce lipid peroxidation by preventing or slowing onset of necrosis inturn improving vascularity. The increased production of reactive oxygen species during injury results in consumption and depletion of the endogenous scavenging compounds such as superoxide dismutase, catalase, and glutathione peroxidase. Flavonoids may have an additive effect to the endogenous scavenging compounds.[41] The delay in wound healing is mostly due to insufficient or excessive fibroblast activity. Inhibition of fibroblast growth by flavonoids such as apigenin could be beneficial for the treatment of skin injury.[42] Flavonoids like rutin, naringin and quercetin protect DNA damage induced by ultraviolet radiation.[43] Quercetin is reported to be useful in healing after renal transplantation.[44] Quercetin showed reduction in inducible form of colonic NO synthase with a reduction in macrophage and granulocyte infiltration in the inflamed tissue of experimental colitis.[45]

Galvez *et al*.[46] have demonstrated the anti diarrhoetic activity in a lyophilized decoction of *E. hirta* whole plant and the active constituent identified was as quercetin 3-rhamnoside (quercitrin). Water extract of *E. hirta* reduces cartilage degeneration in arthritis[9] along with potential anti inflammatory effect reported by Lanhers *et al*.[7] and Martinez-Vazquez *et al*.[8] Phenolic and flavonoid content of *E. hirta* showed potential in-vitro anti oxidative activity.[12] The antioxidant activity of *E. hirta* was comparable with that of ascorbic acid and found to be does dependent. Kandalkar *et al*.[10] reported free radical scavenging activity of *E. hirta* leaves and isolation of active flavonoid myricitrin. Chen[47] reported isolation of gallic acid, quercitrin and myricitriu from leaves of *E. hirta*. Nine Phenolic and flavonoid compounds were isolated and identified as scopoletin, scoparone, isoscopoletin, quercetin, isorhamnetin, pinocembrin, kaempferol, luteolin and gallic acid from the aerial part of *E. hirta*.[48]

Herbs are an excellent alternative to antibiotics in the treatment of infectious diseases, with wider antibacterial effects as well as various antifungal and antiviral actions.[37] Topical application of antimicrobials is essential to enhance normal healing process and prevent infection. *E. hirta* is reported to have excellent antimicrobial as well as antifungal and antiviral properties which may have contributed greatly towards wound healing.[3-6] Plants like *Centrosema pubescens*, *Alternanthera senssilis*, *Acalypha indica*, *Morus alba*, *Ficus bengalensis*, *Tridax procumbence* etc are known to have potential antimicrobial activity contributed to wound healing property.[37,49,50]

The observed enhanced wound contraction effect of *E. hirta* and epithelisation could possibly be made use of clinically in healing of open wounds. However confirmation of this suggestion will need well designed clinical evaluation. The results of the present study indicates that 600 mg/kg dose of *E. hirta* flavonoid fraction possess significant wound heal promoting activity. *E. hirta* contains high amount of flavonoids, which was separated to screen wound healing activity looking in to the potential anti oxidation ability. The potential scavenging effect might be one of the important components of wound healing property enhancing antioxidant enzyme level in granuloma tissues along with contribution of supplementary anti inflammatory, reduction in macrophage and granulocyte infiltration, reduces cartilage degeneration and antimicrobial effect.

CONCLUSION

The present study outcome provides strong evidence for would healing property of *E. hirta* against excision, incision and dead space wound. Conduction of scientific studies on traditional systems of medicine to validate pharmacological activities by screening phytochemical constituents responsible for effect of plant is the need of the time. *E. hirta* has reported antimicrobial, antifungal, antiviral anti inflammatory, anti arthritic, antioxidant and anti anaphylactic effect by number of researchers along with presence of polyphenolic and flavonoid compounds *viz*. myricitrin, quercitrin, quercetin, kaempferol, luteolin and gallic acid all indicating potential wound healing

property. This study reports wound healing effect of *E. hirta* flavonoids containing quercetin, gallic and ferulic acid indicating phytopharmacological correlation and mechanistic approach. The investigation of wound healing activity of *E. hirta* flavonoid indicates that this natural product could be exploited to discover some novel wound healers.

Acknowledgement: The authors are very thankful to management of Radharaman College of Pharmacy, Bhopal, for their encouragement and providing facilities to carry out this work.

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Source of Support: Nil, **Conflict of Interest:** None.