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Comparison of different extracts leaf of *Brassica juncea* Linn on wound healing activity

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ABSTRACT

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care. Brassica juncea is a cruciferous cormophyte medicinal plant. Scientific validations are being made globally to get evidences for traditionally used this plant. Despite its popular usage, no study has been published concerning its wound healing activity till date. Based upon this the study was screened the wound healing activity of different leaf extracts of Brassica juncea Linn in excision wound model in albino rats. Phytochemical constituents were also evaluated. Among different extracts, the aqueous extracts showed 94.94% maximum percentage of healing compared to control. All the extracts are found to be extremely statistical significant and comparable with control. Further wound healing activity was found to be better than standard treated group which may be attributed to the faster action of the active phytochemical constituent.

Keywords: *Brassica juncea* Linn, Wound Healing, Albino rat.

INTRODUCTION

Wound healing process is a complex series of events that begins at the moment of injury and can continue for days to months to years, depending upon the size of injury. It involves a complex interaction between epidermal and dermal cells, the extra cellular matrix (ECM), controlled angiogenesis (the physiological process involving the growth of new blood vessels from pre-existing vessels) and plasma-derived proteins (such as fibrin and immunoglobulins) all coordinated by an array of cytokines and growth factors. This dynamic process is classically

divided into following overlapping phases "Inflammation, Proliferation, Maturation or Remodeling phase" [1].

Before the inflammatory phase is initiated, the clotting cascade (involves thromboplastin) takes place to obtain hemostasis, or stop blood loss by way of a fibrin clot. When tissue is first wounded, blood comes in contact with collagen, triggering blood platelets to begin secreting inflammatory factors. Glycoproteins on the platelet's cell membrane allow them to stick to one another and to aggregate and forming a mass [2]. This is the main structural support for the wound until collagen is deposited.

Now, the key phase of wound healing begins i.e. contraction. At first, contraction occurs without myofibroblast involvement. Later, fibroblasts, stimulated by growth factors, differentiate into myofibroblasts. Myofibroblasts, which are similar to smooth muscle cells, are responsible for contraction [3]. As the actin in myofibroblasts contracts, the wound edges are pulled together. These events signal the onset of the maturation stage of wound healing. The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part.

Traditional and folklore medicines play an important role in health services around the globe. Plant, as illustrated throughout the history of civilization have served as the major source of medication for the treatment of human ailments. More than 80% of the world's population still depends upon traditional medicines for treatment of their ailments [4], especially for wound management [5] as they provide a moist environment to encourage the establishment of suitable conditions for wound healing. About 70% of the wound healing ayurvedic drugs are of plant origin, 205 of mineral origin and the remaining 10% consisting of animal products and these drugs are stated to be effective in different conditions such as *vrana* (wound or ulcers), *Nadivrana* (sinuses), *Vidradhi* (abscess), visarpa (erysipelas), upadamsha (syphilitic ulcers), *Vranjakrimi* (maggots in wounds), *Dustavrana* (septic wounds), *vranashotha* (inflammatory changes of wounds), *Vranavisha* (cellulitis), *Ugravrana* (purulative ulcer), *Netravrana* (hordeolum or stye sepsis), *Pramehapidaka* (diabetic carbuncle) and *Bhagandara* (fistula-in-ano) [6, 7]. India is a tropical country blessed with vast natural resources and ancient knowledge for judicious utilization. However, in order to make these remedies acceptable to modern medicine, there is a need to evaluate them scientifically in order to identify their active principles and understand their mechanism of action.

The family Brassicaceae (=cruciferae) consists of 350 genera and about 3500 species and includes several genera like *Camelina*, *Crambe*, *Sinapis*, *Thlaspi* and *Brassica*. The genus Brassica is the most important one within the tribe Brassiceae, which includes some crops and species of great worldwide economic importance such as *Brassica juncea* L, *Brassica oleraea* L. *Brassica napus* L and *Brassica rapa* L. The same species can be utilized for several uses according to different forms or types. The genus is categorized into oilseed, forage, condiment and vegetable crops by using their buds, inflorescences, leaves, roots, seeds and stems [8].

Indian mustard (*B. juncea* L. Czern and Coss) popularly known as rai, raya and laha is one of the most important oil seed crops of the country and its occupies considerably large acreage among

the brassica group of oil seed crops. India stands first both in acreage and production of rapeseed and mustard in Asia. In India, mustard and rape seed are being grown largely in states like Uttar Pradesh, Rajasthan, Haryana, Assam, Gujarat, Punjab, west Bengal, Madhya Pradesh and also some states of south region [9]. *Brassica juncea* is erecting much branched 3' to 6' high annual plant with slender and tapering root. The stem branches from the axil of the fourth or fifth leaf upward. Lower leaves petioled, green, sometimes with a whitish bloom, ovate to obovate, variously lobed with toothed, scalloped or frilled edges, lyrate-pinnatisect, with 1-2 lobes or leaflets on each side and a larger sparsely sectose, terminal lobe; upper leaves sub entire, short petioled, 30-60 mm long, 2-3.5 mm wide, the fruit is siliqua. The pods are bilocular with a false septum between two halve [9]. Pharmacological activities of *Brassica juncea* has reported by various researchers. Leaf extract of *Brassica juncea* has studied to treat diabetic cataract [10], antioxidant activity both *in vitro* and *in vivo* [11], anti-nociceptive, anti-hyperglycemic activity [12] and hematological studies [13]. Leaf extracts of *Brassica juncea* significantly prevented the development of insulin resistance in rats fed fructose-enriched diet [14]. Anticancer activity has reported on isolation of new compound from leaf extract of *Brassica juncea* [15]. The pharmacological effects of mustard oil have a great deal of interest. The essential oil of *B juncea* has very high application value and can be used to suppress the growth of microorganism in seafood, such as *Helicobacter pyheri* and *Vibrio parahaemolyticus*. It also shows inhibitory effects on growth of bacteria that cause food poisoning and fungi. The oil exhibits significant inhibitory activities against *Aspergillus niger, A. flavus, Trichoderma viride, Candida albicans, C. utilis, C tropicalis, Cryptococcus neoformans, Trichosporon mucoides, Trichophyton tonsrans and Geotrichum capitatum*. Moreover the oil shows inhibitory effect on tumor cells and is effective in anti-platelet and anti cancer. Considering there is a lot of pharmacological activities have been reported till now because of this plant is very common in use. Till date there is no wound healing activity has been reported on *Brassica juncea*. With the broad aim, our present study was to investigate the wound healing activity of leaf extracts of *Brassica Juncea* on experimental rat model.

MATERIALS AND METHODS

Drugs and Chemicals

All the drugs used in this study were of pharmaceutical grade. Povidone-Iodine Ointment (5% w/w) was purchased from (Cipla Ltd, Mumbai, India). All other reagents and chemicals used in the study were of analytical grade.

Plant Material

Leaves of *Brassica juncea* were collected in the month of September 2010 from Botanical garden, Punjab University, Chandigarh, India. The specimen plant (NISCAIR/RHMD/Consult/-2010-11/678/276) was identified with the help of literature and authenticated by Dr. H. B. Singh, Scientist F & Head, Raw Materials Herbarium and Museum, N.I.S.C.A.I.R, New Delhi, India. The fresh plant material were cleaned with distilled water to dry at 35º-40ºC for 10 days and, pulverized in electric grinder and the powder was passed through sieve No.60 and used for further extraction.

Preparation of extracts
The fresh leaves were collected and dried under shade. The leaves were powdered (coarse). This powder was packed into Soxhlet column and extracted with petroleum ether (60 - 80ºC) for 24 hrs. The same marc was successively extracted with chloroform (50 - 60ºC) and later with ethanol (68 - 78ºC) and water (70 - 90ºC) for 24 hrs. The extracts were concentrated on water bath (50ºC) and subjected to soxhlet using petroleum ether, chloroform, ethanol and water respectively. The extracts obtained were evaporated to get a powdery mass. The yield of different extracts was calculated. The powder extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extracts [16].

Gel formulation
A 20% w/v gel of each extract (petroleum ether, chloroform, ethanol, aqueous) was formulated using Cabopol 940 in the concentration of 5%.

Animals
Adult male wistar albino rats weighing about 180-200g were used with the approval of the institute animal ethics committee (MMCP/IEC/10/59 Reg no. 828/ac/04/CPCSEA-Reg. Dt. 16.06.2004). The animals were housed under standard conditions of temperature (24±28°C) and relative humidity (60-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (Lipton India, Ltd) and water a*d-libitum*

Acute dermal toxicity – fixed dose procedure
The acute dermal toxicity study was carried out in Sprague Dawley rats by “fix dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No.434. Extracts were applied topically on rats at a dose level of 1000-2000 mg/kg.

Grouping of Animals
Animals were divided into 6 groups, each group containing 6 rats as follows:
Group 1: Positive Control group
Group 2: Treated with Standard Povidone-Iodine Ointment (5% w/w)
Group 3: Treated with Petroleum ether extract (200mg/kg)
Group 4: Treated with ethanolic extract (200mg/kg)
Group 5: Treated with chloroform extract (200mg/kg)
Group 6: Treated with aqueous extract (200mg/kg)

Wound Healing Activity [17, 18]
Animals were anesthetized (light ether) prior to and during creation of the wounds according to the method of Morton and Malone. The hairs on the skin of back surface of the animals were removed by wiping with a suitable depilatory with help of a cotton swab. A circular wound 78sqmm was made on depilated dorsal thoracic region of animals by cutting the skin of the animals by cutting the skin of the animals by using forceps and scissors. The entire wound was left open. The observation of percentage wound closure was made on day 1^{st}, 3^{rd}, 6^{th} and 9^{th} days post wounding days. The area of the wound was marked by placing a transparency sheet over the wound. The wound areas recorded were measured in square millimeter by using graph paper. This was taken as the initial wound area healing. The treatment was done topically in all the

cases. In the present study no animal showed visible signs of infection. The percentage protection was calculated on the 9th day by using formula.

$$\text{Percentage wound Closure} = \frac{\text{Initial Area of wound - } n^{th} \text{ day area of wound)}}{\text{(Initial area of wound)}} \times 100$$

Statistically analysis

Data were expressed as mean ± SEM of six observations and statistically assessed by two ways analysis of variance and the group means were compared student's *t*-test on statistically software program, SYSTAT 10.6. A probability of $p<0.05$, $p<0.01$ and $p<0.001$ were considered as significant.

RESULTS

The preliminary phytochemical analysis of the crude extracts (pet ether, ethyl acetate, chloroform and ethanol) of *Brassica juncea* indicated the presence of flavonoids, tannins, alkaloids, phenolic compounds, volatile oils and terpenoids. The TLC studies carried out showed the green and yellow colour spots except pet ether not showed green colour (absence of terpenoids) (data not shown). This study was carried out in order to verify the folkloric claims about *Brassica juncea* on a scientific platform. Circular excision wound models were employed for assessing the *in vivo*

Wound healing activity of this medicinal plant. A better healing pattern with complete wound closure was observed in rats treated within 9 days. The studies on excision wound model reveals that all the five groups showed decreased wound area from day to day. However, on 9th day post wounding day, group-1 animals showed 10.24% of healing (which may be due to self immunity of the animals) where as group-2 treated animals showed 82.21% healing. On the other hand among different extracts, the aqueous extracts showed 94.94% maximum percentage of healing (Table 1). All the extracts are found to be extremely statistical significant and comparable with control. The pictures (figure 1) clearly showed the wound healing effect of different extracts of *Brassica juncea* on rats.

DISCUSSION

Wounds are referred to as disruption of normal anatomic structure and function. Skin wounds could happen through several causes like physical injuries resulting in opening and breaking of the skin [19]. The most common symptoms of wounds are bleeding, loss of feeling or function below the wound site, heat and redness around the wound, painful or throbbing sensation, swelling of tissue in the area and pus like drainage [20]. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen composed of amino acid is the major component of extra cellular which gives strength and support. Research on wound healing agents is one of the developing areas in modern biomedical sciences, and many traditional practitioners across the world particularly in countries like Indian and china have valuable information of many lesser known hitherto unknown wild plants for treating

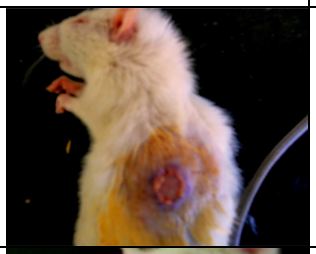
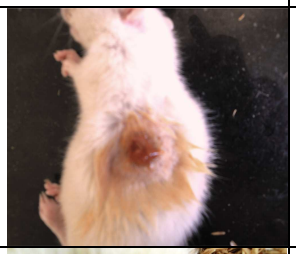
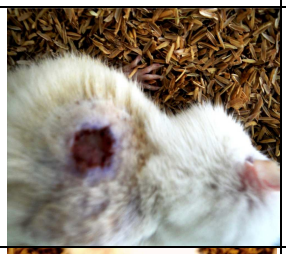
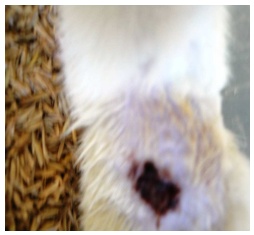
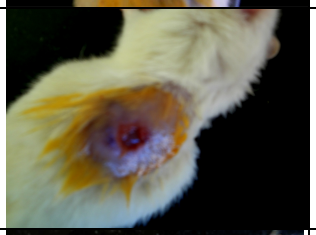
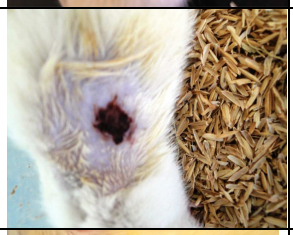
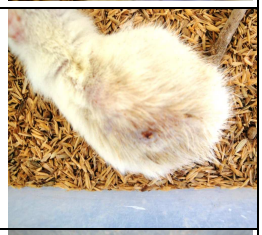
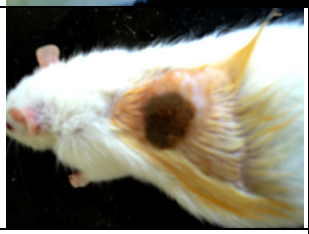
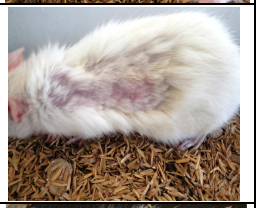
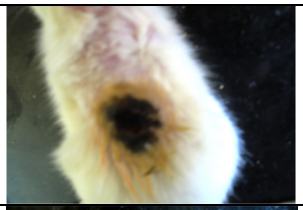
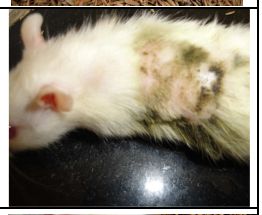
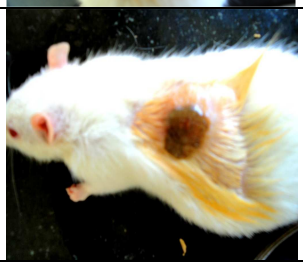
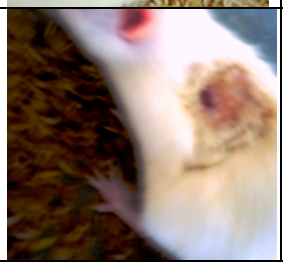
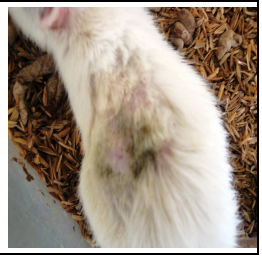
wounds and burns. Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wounds related disorders. *B. juncea* is one of the common traditional plant which are used mostly but unfortunately no researchers till now studied the wound healing activity on this plant. So the present investigation describes potential wound healing capacity of different leaf extracts of *B. juncea* in infected rats. The study revealed that among different extracts, aqueous extract of *B. juncea* showed maximum healing activity and found to be extremely significant compared to control group. The results clearly showed that extracts of *Brassica juncea* showed better wound healing activity than standard treated group. No doubt mostly allopathic medicine shows faster and good wound healing activity than herbal drugs. But in our case, the doubt is very clear the *B. juncea* extracts shows better protection in wound healing activity due to active chemical constituents. However we can assume that the protective factor in this study is due to presence of alkaloids, flavonoids, saponins, tannin and terpenoids. The process is so complex we can assume the mechanism that the reconstruction of the damaged tissue requires the co-ordinated action of a large number of biochemical systems, the nature of which depends on the presence or absence of contaminating toxins in the wound. The flavonoids counteract further decomposition of connective tissue (by collagenases and elastase) and also inhibit PG COX which produces eicosanoids that via a plasma membrane receptor and a signal chain induce the expression of protease genes [21]. The flavonoids are known to reduce platelet aggregation. Cell damage and the viscous metamorphosis of platelets liberate all know inflammation mediators. The wound is covered by epithelial cells due to the interaction of the latter with collagen IV. This process has a mitogenic effect on these cells which within 1 hr begin to replicate. The zone of proliferation at the edge of the wound progresses at a rate of 1mm/day and at first forms a thin layer which subsequently thickens, within 12 days, angiogenic factor, like the basic form of capillaries that improve the supply of nutrients to the wound [22]. This could be the reason for pro-healing activity of this plant. This enhanced wound contraction effect of *Brassica juncea* and epithelization could possibly be made use of clinically in healing open wounds. However confirmation of this suggestion will need designed clinical evaluation.

Table 1: Effect of topical application of different leaf extracts of *Brassica juncea* on excision wound model

Day→ Group ↓	Day 1	Day 3	Day 6	Day 9
Group 1 (Positive Control)	78.57±12.5 (0%)	78.57±12.5 (0%)	76.64±11.3 (2.45%)	70.50±10.6 (10.27%)
Group 2 (Standard Treated)	79.32±12.8 (0%)	44.19±8.4*** (44.28%)	25.97±10.5*** (67.25%)	14.19±3.1*** (82.11%)
Group 3 (Pet. ether extract Treated)	78.11±10.5 (0%)	35.79±6.4*** (54.18%)	23.76±9.3*** (69.58%)	8.29±2.4*** (89.38%)
Group 4 (Ethanolic extract Treated)	78.66±11.2 (0%)	38.50±7.3*** (51.05%)	23.76±9.3*** (69.79%)	7.07±2.7*** (91.01%)
Group 5 (Chloroform extract Treated)	79.42±13.1 (0%)	33.19±8.4*** (58.20%)	25.97±4.8*** (67.30%)	5.94±2.2*** (92.52%)
Group 6 (Aqueous extract Treated)	78.57±12.5 (0%)	47.19±6.8*** (39.93%)	28.28±6.6*** (64%)	3.97±1.5*** (94.94%)

Average Wound Area (mm^2), Values are Mean ± SEM (% wound healing)
Data was analyzed by one-way ANOVA followed by Tukey's t test
Statistically significant value:
**** $P \leq 0.0001$*

Figure 1 Comparison of wound site by excision wound model in control, standard and extracts treated group

Day→ / Group ↓	1	3	6	9
Group 1 (Positive control)				
Group 2 (Standard)				
Group 3 (Pet. ether)				
Group 4 (Ethanolic)				
Group 5 (Chloroform)				
Group 6 (Aqueous)				

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