Contents list available at Journal of Science Letters

Journal of Science Letters

journal homepage: http://www.scienceletters.org/

Standardization and Pharmacological Investigation of Different Extracts of *Brassica juncea* Seed for Wound Healing Activity

Sanjay Kumar[1]* and Ranjit Singh[2]

[1]School of Pharmacy, Career Point University Hamirpur HP-176041 India
[2]Shobhit University, Saharanpur, Uttar Pradesh – 247341, India

ARTICLE INFO

Article History:
Received 16 November 2021
Revised 29 December 2021
Accepted 10 January 2022
Available Online 21 January 2022

Keywords:
Brassica juncea,
burn,
flavonoid,
excision wound model,
incision wound model,
tensile strength

ABSTRACT

Majority of the global population from developing countries relies on herbal medicines for cure of diseases. The ability to sense the environment and sustain physico-chemical and thermal homeostasis is dependent on our skin. Healing process is the mechanism of repairing the skin and other soft tissues after an injury. Brassica juncea has traditionally been used for its antibacterial and antioxidant properties. The current research was intended to investigate the wound-healing behaviour of B. juncea in order to generate comprehensive scientific evidence. Excision and incision wound models were used to test the wound-healing effectiveness of aqueous, ethanolic, and petroleum ether extracts of B. juncea. Physical analyses of the crude compound were carried out along with the quantitative analysis of phenolics and flavonoids. The rate of wound contraction, the duration of full epithelialization, and the tensile strength of the incision wound were investigated. The three extracts from B. juncea seeds were noted to heal the wound, as evidenced by a reduction in epithelialization time, an improvement in wound contraction rate, and increased skin-breaking ability. The ethanolic and aqueous extracts have characteristics that make them effective in wound- healing action as compared to a placebo control, according to the current report.

1. Introduction

About eighty percent of the global population, especially in developing countries, uses herbal medicines for primary health care. Herbal drugs have the benefits of stability, potency,cultural appropriateness, and reduced side effects. Since their chemical components are involved in the biochemical processes of living flora, they are thought to be more compatible with the human body (Ekor, 2014). India, as one of the world's twelve mega-biodiversity countries, exports herbal raw resources valued between $100 and 114 million dollars per year (Oyebode *et al*., 2016).

The skin is the largest organ which plays a major role in shielding the human body from unsafe foreign agents including microorganisms, contaminants, and chemicals, which can weaken the body's protective shield when injuries or wounds occur (Chuong *et al*., 2002). Every year, millions of people all over the world suffer both from acute and chronic skin trauma, with about 37 million people suffering from chronic wounds (Sen *et al*., 2009). The epidermal integrity of the skin would be lost in a burn wound caused by tissue damage following exposure to heat from some direction. Burns are described by extreme skin damage that results in the death of the infected skin cells. That may be mild medical issues or life- threatening situations. Burns killed approximately 300,000 people a year across the world, and they can happen to someone of any age or gender, in both developing and underdeveloped nations (Bunman *et al*., 2017). For older people, smoking and using an open flame are the primary sources of burn injuries. Children's burn injuries are most often caused by drenching. Infants and the elderly are the most vulnerable to burn injuries (Peden *et al*., 2008; Ye and De, 2017).

Wound healing is the mechanism of physical tissue healing that includes soluble mediators including cytokines and growth factors, blood cells like platelets and white blood cells, extracellular matrix, and parenchymal cells. Burn therapy is considered symptomatic due to the wide range of etiological causes. As a result, surgical tools have been used to ease pain in the affected region, speed up the healing process, and extend the time between lesions. In terms of the completeness of healing of burns and chronic wounds,

*Corresponding Author: Sanjay Kumar
E-mail Address: diwansanjay1981@gmail.com
DOI: 10.46890/SL.2022.v03i01.004

traditional collagen sheet dressings do not outperform conventional dressings (Singh *et al*., 2011). As a result; medicinal plants are an essential part of the human health-care system for wound management. Different flora contains a wide range of plants, each of which is known for its wound-healing abilities and medicinal properties. However, there are many plants whichhave not been investigated and whose phytotherapeutic qualities are unknown, one of whichis *B. juncea*, which is yet to be identified for its wound healing activity.

B. juncea is an important socioeconomic plant that has been valued in India for centuries for its nutritional and medicinal properties. It is a perennial annual herb found throughout the world, mostly in Africa, Russia, Sri Lanka, Japan, and India (Rahman *et al*., 2018). Various phytochemicals are found in *B. juncea*, including nutrients, sulfur-containing glucosinolates (sinigrin, glucoiberverin, glucobarbarin, gluconapin, glucoalyssin, neo glucobrassicin), polyphenols (gallic acid, caffeic acid vanillin rutin, naringin, chlorogenic acid catechin), and volatile components (allyl isothiocyanate, 3-butyl isothiocyanate, neo glucobrassicin, neo glucobrassicin, tetradecane) (Tian and Deng, 2020). *B. juncea* is a stimulant, emmenagogue, and antioxidant that are used to treat diabetic cataracts, fever, nausea, backache, arthritis, paralysis, and edema of the lungs and liver. *B. juncea* seed is used to treat asthma, foot pain, lumbago, tumors, urinary diseases, inflammation or haemorrhage, and rheumatism in China and Korea (Khandayataray and Murthy, 2019; Le *et al*., 2020).

However, no evidence of wound healing action of *B. juncea* seed was found in the literature. Therefore, the current research was designed to standardize *B. juncea* seeds and investigate the anti-inflammatory and wound-healing properties.

Materials And Methods

Ethanol was procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. Phloroglucinol, hydrochloric acid and other chemicals were procured from standard sources.
Collection, processing and authentication

B. juncea mature seeds (1 to 1.2 kg) were collected from a local market in Hamirpur District, Himachal Pradesh. The taxonomist at Herbal Health Research Consortium Pvt. Ltd., East Mohan Nagar Amritsar, India authenticated the plant with specimen no R.S.-152 and seeds specimen as *B. juncea* (Local name: Sarson) of the Brassicaceae family. For future reference, voucher collections of the plant were stored in the Herbal Health Research Consortium Pvt. Ltd., herbarium. The seeds were then cleaned with purified water and shade dried for 7 days at room temperature in a ventilated dark spot. The dried seeds were stored for the further processing in an airtight poly pack bag.

Organoleptic evaluation and drying

The organoleptic properties of *B. juncea* seeds were assessed, including size, form, colour, odour, taste, fracture, and soil. Seeds were semi-dried in the air for 12 hours during the day (without being exposed to sunlight) and for 72 hours during the night period. The semi-dried seeds were then air dried for another 8 to 12 days at room temperature and 45 percent relativehumidity in a dry ventilated area. When a constant weight of seed was obtained, it was assumed that no moisture was present in the rudimentary seeds, indicating that the drying process was complete. At last, the amount of moisture loss was calculated.
Physical evaluation

Physical requirements for the coarse powder of *B. juncea* seeds were developed. These are usually ineffective for crude drugs, but they may support in assessment, especially in terms of moisture content, foreign organic matter, volatile oil content, ash (total ash, acid insolubleash, water soluble ash, and sulphated ash), and extractive value (Evans, 2004, Kokate, 2007; Kokate *et al.,* 2007; Khandelwal, 2008).

Extractive value

In a stoppered 250 ml conical flask, 5 g of powdered seeds was macerated for 24 hours with 100 ml of each solvent (water and ethanol respectively). The flask was regularly shaken for the first six hours, and left to stand for another eighteen hours. Following filtration, 25 mL of filtrate was transferred to a weighted, thin porcelain dish and dried in ahot air oven (Remi RDHO-80, Remi Elektrotechnik Ltd., India) at 100 °C. The extracts were weighed after cooling in a desiccator. The extractive percentage (w/w) was measured usingair dried drugs as a reference.

Extraction of seeds

The Soxhlation process was used for extraction of crude seeds of *B. juncea* using water and ethanol (analytical grade) as solvents. The solvents were chosen based on their polarity in decreasing order. In a Soxhlet extractor, coarse powders were packed (Model no 3840013, Borosil Glass work Limited, Mumbai, India). A total of 250 to 300 g coarse powders were extracted separately with 500 ml of solvents. The yield of the extract was determined using the formula below, regardless of the cumulative volume of crude drug in coarse powder form taken into the extractor.

$$\text{Yield of extract (\%)} = \frac{W_1}{W_2} \times 100$$

Preliminary phytochemical screening

According to normal protocols, the aqueous and ethanolic extracts of the seeds of *B. juncea* were subjected to qualitative chemical tests for the identification of multiple chemical constituents (Doss, 2009; Venkataswamy et al., 2010; Vijayaram et al., 2016).

Microscopy of powdered crude seeds

Separately, coarse powdered crude seeds of *B. juncea* were put on a clean and dry glass slide. A few drops of chloral hydrate were added to the powder. The slides were placed on a Bunsen burner until chloral hydrate boiled. After 5 minutes of heating, the slides were cooled to room temperature. A few drops of phloroglucinol and HCl were added to dye the samples (1:1). A compound microscope (Esaw SM-02 Student Compound Microscope, ESAW, India) was used to examine the stained samples at a resolution of 100x. The findings were then noted (Alam and Us Saqib,

2015).

Quantification of phytoconstituents

The quantification of phenolic and flavonoid compounds in aqueous and ethanolic extracts of *B. juncea* seeds was done using the methods as described in Ayurvedic Pharmacopoeia (The Ayurvedic Pharmacopeia of India, 2001).

Determination of phenolic content

The Folin-Ciocalteu reagent was diluted 1:10 (v/v) with sterile water and incubated for 5 minutes at 25 °C before adding and mixing 0.8 ml of 7.5 percent (w/v) sodium carbonate. A
0.2 ml aliquot of diluted extracts was combined with 1 ml of the above developed Folin- Ciocalteu reagent and left to complete the reaction for a few minutes. After that, the reaction mixture was incubated at room temperature for 1 hour. A UV-Visible spectrophotometer (UV-2600i, Shimadzu, Japan) was used to determine the absorbance of the color solution at a wavelength of 765 nm. As a standard phenolic drug, gallic acid was used. A standard curve was plotted using the absorbance versus concentration data. The regression coefficient derived from the standard curve was used to calculate the overall phenolic content in aqueous and ethanol extracts, which was expressed in µg/ml.

Determination of flavonoid content

0.5 mg of the extracts was combined with 1.5 ml pure water followed by addition of 0.75 ml sodium nitrite solution (5%), and the mixture was kept aside for 5 to 6 minutes. After that, 0.2 mL of a 10% $AlCl_3$ solution was added and left to rest for another 5 minutes. Finally, 0.5 ml of 1M NaOH was added, and the volume was made up to 2.5 ml by adding purified water.As a common compound, quercetin was used. Taking five standard quercetin solutions of concentration 10, 20, 30, 40, and 50 µg/ml, a standard curve of quercetin was prepared. AUV-Visible spectrophotometer was used to assess the absorbance of the prepared final testand normal solutions at a maximum wavelength of 510 nm. The regression coefficient derived from the standard curve was used to calculate the total flavonoids content in the extract, which was expressed in µg/ml (Chandra *et al*, 2014; Sankhalkar & Vernekar, 2016).

Acute toxicity studies

The OECD-423 recommendations were followed while conducting the acute oral toxicity analysis (OECD, 2002). Individual aqueous and ethanolic extracts were assessed for toxicity in laboratory animals at doses of 5, 50, 300, and 2000 mg/kg body weight of Wister rats. For fourteen days, toxic signs and magnitude, as well as their onset, progression, and reversibility, were detected and reported as a function of dosage and time. Animals which died during the observation phase, as well as those which survived at the end, were autopsied (Tunner, 2009; Dinda and Mukharjee, 2009; Mathur, 2009).

Experimental animals

In this study, healthy wistar rats of either sex weighing 180 to 250 g were used. They were held in animal houses under normal conditions of temperature (25±2 °C) and relative humidity (45-55%). They were given a normal pellet diet and free access to water. For 15 days, all of the animals were closely supervised and cared in compliance to the CPCSEA recommendations. Animals were kept in polypropylene containers, and all procedures on them were carried out in an aseptic environment. The report was reviewed and approved by the Institutional Animal Ethics Committee (Protocol no.: CPCSEA/LIPH/2019/13).

Anti-inflammatory activity

The anti-inflammatory potential of the extracts was tested by three different research models using the carrageenan-induced rat paw oedema process (Mujumdar et al., 2000; Chen et al., 2007; Mathur, 2009). For different therapies, twenty-four rats were split into eight groups of six rats each. Group I (normal control) was treated with 2 ml/kg body weight saline water, group II (standard control) was treated with 5 mg/kg body weight diclofenac sodium, group III & IV animals were administered with aqueous extract of seed of *B. juncea* (BJAE); i.p at doses of 50 & 150 mg/kg body weights, group IV & VI animals were administered with ethanolic extract of seed of *B. juncea* (BJEE); respectively.

The inflammatory agent carrageenan was given in solution form with usual saline water as the vehicle in all groups except normal control group. To induce oedema, 0.05 ml of a 1 percent solution of carrageenan was injected subcutaneously into the plantar area of the right hind paw 30 minutes after the above injection. The oedema was measured as an increase in paw thickness as a result of the carrageenan treatment. Using a Plethysmometer, the paw volume was determined at the start and at 2, 3, 4, and 6 hours after carrageenan injection. The following equation was used to measure the percentage inhibition of paw thickness (Parmer & Prakash, 2006; Patil et al., 2011).

$$\text{Inhibition of Paw Thickness (\%)} = 1 - \frac{V_t}{V_c} \times 100$$

Preparation of the ointment

The ointment of the seed extracts was made with a standard ointment base (Table 1). The aqueous and ethanolic extracts were taken individually on a clear, dry, and sterile tile and mixed with melted soft paraffin in a water bath at 70°. The surfactants sorbitan monolaurate and tween 80 were dispersed in the aqueous and oil phases respectively. Quantities of different plant extracts were accordingly mixed together to form the aqueous phase. The aqueous phase was slowly added to the oil phase with continuous stirring. On addition of all the aqueous phase the mixture was mixed for another 5 min before and stored in an airtight glass bottle for further use in a clean, dry, and sterile setting (Builders et al., 2013).

Excision wound model

The rats were divided into four groups, each with six rats (Table 2). For fourteen days, the animal in groups III to IV were given individual aqueous and ethanolic extracts ointment twice a day at a 12-hour interval.

Experimental procedure

The full thickness excision wound was inflicted in animals using the procedure described in the literature (Rathi & Bodhankar Baheti, 2006; Somboonwang *et al*., 2012). The hairs on the back of the rat were first shaved. Isopropyl alcohol (70 percent v/v) was used to disinfect the shaved patch. Sodium pentobarbital at 60 mg/kg b.w. was used intraperitoneally to anaesthetize the rats in shaved fields. 1 cm diameter cylindrical steel was heated to 100°C in boiling water. This heated cylinder was placed on the rat's shaved area for 6 to 8 seconds before being replaced.

Evaluation of wound healing activity

Daily, the wounds of the treated animals were externally inspected for color, swelling, the consistency of the tissue covering the wound, and the existence of exudates. The wound diameter was measured. The wound healing was calculated using the following equation, which determined the wound closing (%).

$$\text{Wound Closure (\%)} = \frac{\text{IAW} - \text{FAW}}{\text{IAW}} \times 100$$

Where, IAW and FAW are initial and final area of wound.

Incision wound model

The rats were divided into IV groups, each one with six rats (Table 3). Specific aqueous and ethanolic, extracts ointment was applied to the animals in group III to IV twice a day at the intervals of 12 hours for up to eight days (Somboonwang *et al*., 2012).

Experimental Procedure

The ability of various extracts to heal incision wounds was tested. Initially, the animal was anesthetized with xylocaine on the back side. Using a modern sterile Sharpe comb, the paravertebral region's fur was gently shaved. A 3 cm long section of skin corresponding tothe vertebra on the rear portion of the animal was labelled with a marker and incised with a thin surgical needle. Within 1 cm, the incised part of the skin was closed with an interrupted suture. Both operations were performed sterile in part and during the study; no antimicrobials were used locally or systemically (Muscará *et al*., 2000; Menke *et al*., 2007; Boateng *et al*., 2008; Mechesso *et al*., 2016).

The suture was removed from the animal on the eighth day, and therapy with ointment was started on the ninth day as well. The tensile strength of the wound was calculated for all ofthe treated rats on the tenth day using the continuous water flow process. Wet and dry granulation tissue weights were measured on the tenth day.

Measurement of tensile strength

The tensile strength of a wound indicates how well it is healing. It reveals how resistant the restored tissue is to tearing under strain and may reflect the restored tissue's integrity in proportion. On the ninth day after wounding, the sutures were removed, and the tensile strength of the excised tissue was measured with a tensiometer on the tenth day. Wound breaking strength was calculated using this approach as the weight of water per area of the specimen at the moment of wound breaking.
The wet and dry granulation weights of incised tissue were recorded using an automated digital balance after the tensile strength calculation.

Statistical analysis

GraphPad Instat 5.0 version was used to analyse the data (GraphPad, San Diego, CA). The statistical analyses were performed using one-way analysis of variance (ANOVA) and Dunnett's post hoc multiple comparison test. The significance level was set at $P < 0.05$.

Results And Discussion

Burn injury is described as destruction to the tissues of the body produced by fire, toxins, lightning, sun, or irradiation. It is among the most prevalent traumatic complications throughout the globe. Patients with burn injuries needed to focus on wound healing and pathogen avoidance.

Organoleptic properties and drying

The organoleptic properties of *B. juncea* seed is presented in Table 4. The seeds werediscovered to have a pungent odour and a sour taste when crushed which could be related to the presence of certain highly bioactive compounds. This may be attributed to the presence ofsome volatile oils in the seeds. The seed had a smooth texture.

Physical properties

Table 5 shows the data of the physical attributes of the drug. Moisture is one of the most important variables in the degradation of pharmaceuticals and formulations. Low moisture content is usually preferable for better medication stability. The coarse powder of seeds showed moisture content of 3.1 percent. This finding illustrates why this crude drug is less susceptible to microbial decomposition and enzyme - mediated neutralization, implying that it can last better. The low amount of organic materials (0.132 percent w/w) indicates that the crude material is of the utmost clarity and grade conceivable. The ash value is helpful in confirming sample validity and integrity, as well as being significant quality criteria. The volatile oil content was also found to be 21.74 percent. This study confirms the highest purity and quality of the drug. Complete, acid insoluble, water soluble, and sulphated ash values were found to be 4.25, 3.95, 1.85, and 2.45 percent w/w, respectively indicating that the root is the best for medication action and effects.

Extractive values and extraction

The seed powder was found to be reddish brown in colour. The extracts exhibited mild pungent odour. Water and ethanolic had extractive values of 4.732 and 8.774 percent, respectively. The extractive yield of ethanolic extract was the highest, while the extractive yield of aqueous extract was found to be lowest (Table 5). Figure 1 shows the yield

Table 1. Formulation of ointment of selected plant extracts

Components	BJAE (g)	BJEA (g)
Extracts	5	5
Petrolatum	18	18
Glycerol	5	5
Sorbitan Monolaurate	5	5
Tween 80	2	2
Water	15	15

Table 2. Grouping of animals and treatment protocol for excision wound model

Group		Treatment
I (Normal Control)	**Induction of Excision Wound**	Plain ointment base
II (Standard Control)		1% w/w Silver sulfadiazine
III (BJAE 5% w/w)		Aqueous seed extract ointment of *Brassica juncea*
IV (BJEE 5% w/w)		Ethanolic seed extract ointment of *Brassica juncea*

Table 3. Grouping of animals and treatment protocol for incision wound model

Group		Treatment
I (Normal Control)	**Induction of Excision Wound**	Plain ointment base
II (Standard Control)		1% w/w Silver sulfadiazine
III (BJAE 5% w/w)		Aqueous seed extract ointment of *Brassica juncea*
IV (BJEE 5% w/w)		Ethanolic seed extract ointment of *Brassica juncea*

Table 3. The organoleptic characteristics of the seeds

S. No.	Parameter	Characteristics
1	Size (diameter)	0.8 to 1.1 mm
2	Shape	Spherical
3	Color	Reddish brown
4	Odour (crushed)	Pungent
5	Taste	Bitter
6	Surface	Smooth

Table 4. Organoleptic evaluation of the coarse powder of the seeds

S. No.	Parameters	Values in percentage (% w/w)
1	Moisture content	3.112 ±0.17
2	Foreign organic matter	0.132 ± 0.21
3	Volatile oil content	21.74 ± 0.13
4	Total ash	4.255 ± 0.19
5	Acid insoluble ash	3.951 ± 0.23
6	Water soluble ash	1.858 ± 0.12
7	Sulphated ash	2.456 ± 0.28

Table 5. Physical and extractive values of different extracts

S. No.	Specifications	Values in percentage (w/w)
1	Colour	Reddish brown
2	Odour	Pungent
	Extractive value	
3	Aqueous extract	4.732 ± 0.41
4	Ethanolic extract	8.774 ± 0.29

Table 6. Acute toxicity study of *Brassica juncea* seeds extracts using mice model

S. No.	Drugs	Doses (mg/kg body weight)	Toxicity effect					
			3rd day		5th day		7th day	
			Death	MBS	Death	MBS	Death	MBS
1	Aqueous extract	Low (1000<)	-	-	-	-	-	-
		High (> 1000)	-	-	-	-	-	-
2	Ethanolic extract	Low (1000<)	-	-	-	-	-	-
		High (> 1000)	-	-	-	+	-	+

MBS: Mice behaviour status. The + sign represents the presence of death or abnormal behaviour, whereas the - sign represents the absence of death or abnormal behaviour does notoccur.

Table 7. Anti-inflammatory activity of the extracts in Wistar rats by carrageenan induced ratpaw oedema method

Groups	Carrageenan induced rat paw edema volume in mm (% inhibition)				
	Time (h)				
	0 h	2 h	3 h	4 h	6 h
I (Normal Control)	1.76± 0.81	1.84±0.88	1.88±0.64	1.77±0.70	1.75±0.52
II (Standard Control)	1.88± 0.77	1.47±0.86 (21.81)	1.26±1.01 (32.98)	1.13±0.96 (39.89)	1.08±0.73 (42.55)
III(BJAE 50 mg/kg)	1.76± 1.02	1.50±0.79 (14.6)	1.42±0.77 (19.2)	1.35±0.81 (23.3*)	1.23±0.69 (29.9**)
IV (BJAE 150 mg/kg)	1.75± 0.87	1.41±0.90 (19.2)	1.32±0.88 (24.4*)	1.23±0.95 (29.5**)	1.08±0.86 (38.3***)
V (BJEE 50 mg/kg)	1.82± 0.98	1.60±1.01 (11.8)	1.58±0.75 (13.3)	1.52±0.70 (16.1)	1.40±0.93 (22.8*)
VI (BJEE 150 mg/kg)	1.81± 0.61	1.56±0.84 (13.5)	1.33±0.97 (19.7)	1.43±0.61 (26.6*)	1.22±0.81 (32.6**)

Continued..

Mean represents thickness of paw edema in mm. Each value represents mean ± standard deviation (n = 6) with statistical analysis by one-way ANOVA followed by Dunnett's multiple comparison test. * signifies $P < 0.05$; ** signifies $P < 0.01$ and *** signifies $P < 0.001$ when compared with the standard drug.

Table 8. The wound closure value of the seeds extract by excision wound model

Group	Wound Closure (%)					Epithelization Time (Days)
	Days					
	2nd	5th	8th	11th	14th	
I (Normal Control)	11.8±0.74	20.3±0.98	28.8±0.66	39.5±1.02	55.3±0.87	22.5±0.24
II (Standard Control)	31.3±0.44	40.6±0.58	56.9±0.81	66.4±0.69	98.4±0.74	14.5±0.36
III (BJAE 5% w/w)	27.7±0.91	35.5±0.71	51.6±1.03*	62.2±0.99*	95.2±1.01**	16.4±0.64
IV (BJEE 5% w/w)	30.7±0.88	38.8±0.5 7	54.3±0.84*	65.1±0.87*	97.4±0.86**	14.8±0.58

Group I treated with plain ointment base, Group II treated with 1% w/w silver sulfadiazine ointment and Group III to IV were treated with 5% w/w ointment of aqueous and ethanolic extracts of *Brassica juncea* seed. All data were presented as mean ± standard deviation (n = 6) with statistical analysis by one-way ANOVA followed by Dunnett's multiple comparison test. * signifies $P < 0.05$ and ** signifies $P < 0.01$ when compared with the standard drug.

Table 9. The wound healing activity of *Brassica juncea* seeds extracts by incision wound model

Group	Tensile strength(gmm^2)	Tensile strength(%)	Wet granulation tissue weight (g)	Dry granulation tissue weight (g)
I (Normal Control)	228.2±0.98	-	212.4±0.88	67.8±0.58
II (StandardControl)	312.6±1.12	36.98	346.7±1.02	121.2±0.67
III (BJAE 5% w/w)	275.4±0.95	20.67	331.3±0.88	107.4±1.04
IV (BJEE 5% w/w)	283.5±1.05	24.23	340.1±0.89**	111.2±0.66

Group I treated with plain ointment base, Group II treated with 1% w/w silver sulfadiazine ointment and Group III to IV were treated with 5% w/w ointment of aqueous and ethanolic extracts of *Brassica juncea* seed. All data were presented as mean ± standard deviation (n = 6) with statistical analysis by one-way ANOVA followed by Dunnett's multiple comparison test. * signifies $P < 0.05$ and ** signifies $P < 0.01$ when compared withthe standard drug.

Yield Value Extracted by Soxhlation

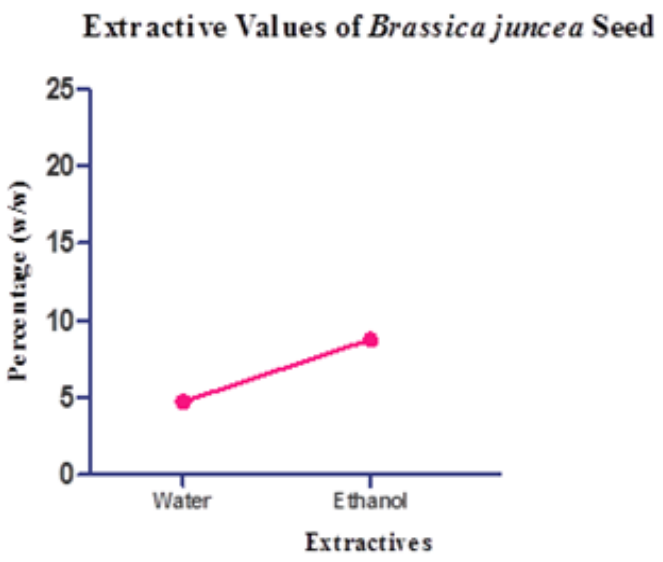

Figure 1. Yield value of various extracts

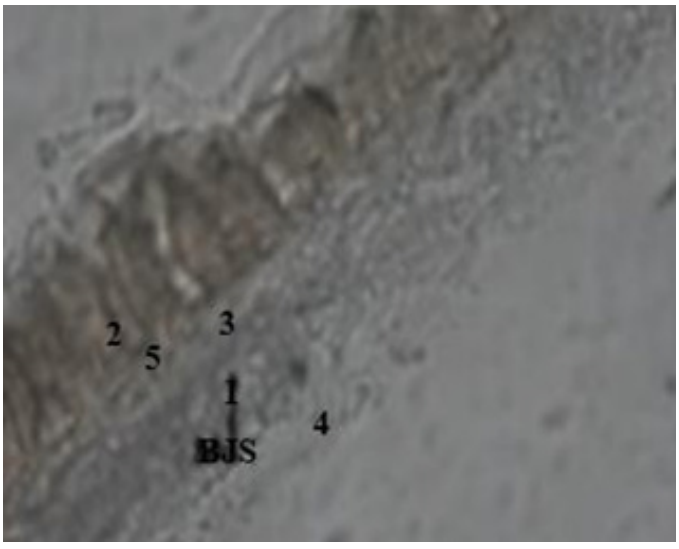

Figure 2. The microscopic study of the seed showing 1: Mucilage; 2: Seed coat; 3: Palisade cells; 4: Tegmen; 5: Testa

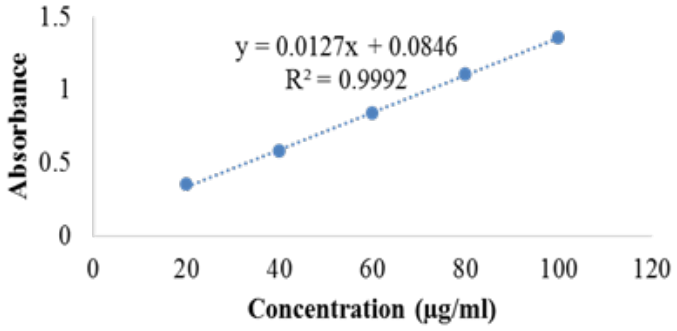

Figure 3. The calibration curve of gallic acid

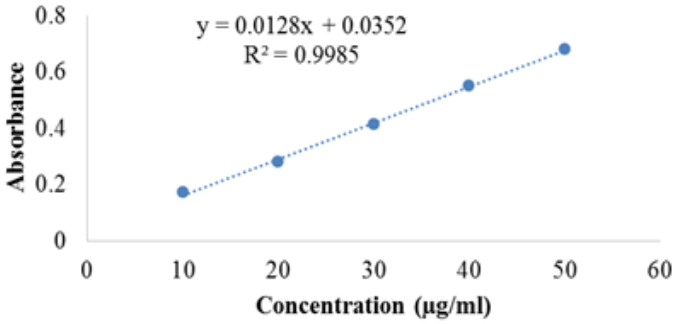

Figure 4. The calibration curve of quercetin

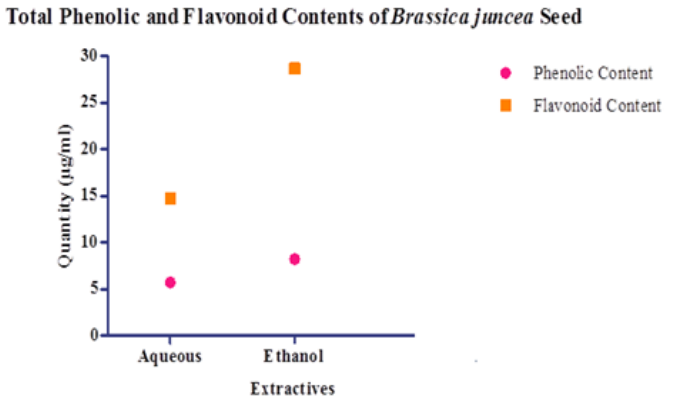

Figure 5. Total phenolic and flavonoid contents of the seeds

value of extracts with aqueous and ethanol as solvents.

Phytochemical screening

The study indicated that the aqueous extract contains sugars, cardiac glycosides, tannins, sterols and steroids, alkaloids, proteins, amino acids, fixed oils, triterpenoids, saponins, phenols, and flavonoids, alkaloids, proteins, amino acids, fixed oils, triterpenoids, saponins and phenols. Alkaloids, enzymes, cardiac glycosides, saponins, fats, amino acids, fixed oils, triterpenoids, phenols, and flavonoids are present in the ethanolic extract of the seeds. Phenolic compounds, such as flavonoids, are well-known antioxidants and a variety of other bioactive molecules which have historically piqued attention owing to their potential advantages for humanhealth, including the treatment and prevention of a variety of illnesses. Free radical scavenging activity has been connected to wound formation, while antioxidants have been associated to wound healing capacity (Tungmunnithum *et al*., 2008). These antioxidant compounds are useful candidate in reducing the causes and consequences of aging skin, skin disorders, and skin injury especially wounds and burns, owing to their biological source and low cytotoxicity (Działo *et al*., 2006).

Powder microscopic study

The seeds (Figure 2) were found to be mildly yellowish brown in color when examined under a microscope. The microscopic anatomical form revealed that the matured seed has released mucilage. Dark reddish brown testa makes up the crop. The cotyledons are present in the oily embryos. Tegmen was also present in the seed.

Quantification of phytochemicals

Increased production of reactive oxygen species, exposure to exogenous oxidant chemicals, or a breakdown in the regulatory systems in our bodies can destroy important macromolecules like DNA, lipids, and proteins, as well as impede wound healing. Natural antioxidants, particularly plant phenolics and flavonoids, have been shown to protect or postpone degenerative illnesses induced by free radicals (Agar *et al*., 2015).
Figure 3 shows the standard curve and regression coefficient for gallic acid solutions. The regression equation was found to be y = 0.0127x + 0.0846, with a regression coefficient of 0.9992. Figure 4 shows the standard curve data and regression coefficient of quercetin standard solutions. The regression equation was noted to be y = 0.0128x + 0.0352, with a regression coefficient of 0.9985. Figure 5 shows the overall phenolic and flavonoid contents of various seeds extracts.

The sum total of phenols and flavonoids in aqueous and ethanol extracts was estimated and represented as gallic acid equivalents (Figure 5). The aqueous and ethanol extracts were found to have overall phenolic contents of 5.72 and 8.21 g/ml, respectively. The phenolic content of the ethanoloic extract was higher and those of the aqueous extract were lower. *B. juncea* seeds contained 14.76 and 28.66 g/ml of total flavonoids in aqueous and ethanol extracts, respectively. The flavonoids content of the ethanolic extract was higher, and those of the aqueous extract were lower. The high levels of phenol and flavonoid detected in *B. juncea* seed extracts were also documented in this investigation that revealed its promise as a burn injury treatment.

Acute toxicity study

The seed extracts of *B. juncea* were determined to be non-toxic at 3000 mg/kg body weightof experimental animals in an experiment conducted according to OECD Guidelines 423. Table 6 shows the findings of an acute toxicity analysis of aqueous and ethanolic extracts. In each category of animals, no major toxic signs or frequency were observed. There was no mention of a death. An acute toxicity analysis in Wistar rats showed no death in any solvent extract at any dosage, confirming that *B. juncea* seeds extracts are non-toxic in the living body. Even at the peak dosage, 3000 mg/kg body weight of mice, no lethality was observed. It was not possible to assess the lethal dose (LD_{50}). Based on the toxicity results, the plant extracts were dosed to 50 mg/kg and 150 mg/kg b.w. for assessment of pharmacological

activities.

Anti-inflammatory activity

Arachidonic acid is degraded after inflammation by pro-inflammatory enzymes such as COX or LOX pathway, which produces prostaglandins and leukotrienes. Inhibition of these pathways might be used to treat a variety of inflammatory and autoimmune diseases. Many phenolic chemicals, such as flavonoids, phenolics, and tannins, are considered to work in the inflammatory cascades by scavenging free radicals or inhibiting pro-inflammatory enzymes (Adebayo *et al*., 2015).

Table 7 shows the anti-inflammatory action of different extracts. The results revealed that *B. juncea* seed extracts have anti-inflammatory activity that is dose dependent and were similar to the standard medication diclofenac sodium. All of the extract doses had lower anti-inflammatory activity than diclofenac sodium, the reference drug. Among various extracts, the aqueous extract was more efficient. The anti-inflammatory properties the extracts were observed in the following order: aqueous > ethanolic extracts. In comparison to the normal treatment, the aqueous seed extract showed potent anti-inflammatory efficacy. This may be attributed to the involvement of influential chemical components in the aqueous extract that function as inhibitors of inflammatory mediators. It can thus be inferred that the seeds have anti-inflammatory efficacy, but not as powerful as standard medicine. The aqueous extract was noted to be the best of the two extracts. All of the two extracts of *B. juncea* seeds had moderate anti-inflammatory effects after 1 hour at both the doses. While theaqueous seed extracts had strong anti-inflammatory efficacy as compared to the standard diclofenac sodium, the dosage of the aqueous extracts was much higher. As a result, the extract must be purified further into a specific chemical entity form, with a lower dosage that may be useful in the effective treatment of inflammation.

Excision wound healing activity

Wound contraction is a crucial step in the healing process that leads to wound closure. As a consequence, physical appearances and wound contraction measurements have become reliable basis for evaluating macroscopic wound healing (Vafi et al., 2016). Table 8 shows the wound healing potential of the extracts using a wound excision model and a rat as an animal model. To mimic available commercial Silver sulfadiazine ointment, the extracts were applied to animals as an ointment. All of the extracts showed wound healing activity. The seed extracts have wound healing activity similar to silver sulfadiazine ointment. Using the statistical approach, it was discovered that the ethanolic extract had extremely potent healing properties ($P < 0.01$), while the aqueous extract had comparative potency to that of the standard drug ($P < 0.05$). On the eighth day of treatment, a substantial wound closure was detected, and on the sixteenth day of treatment, approximately 100 percent healing was achieved. The wound healing action was in the order ethanol > aqueous extracts. The wound healing efficacy of ethanol extract ointment was comparable to that of a standard medication ointment.

The presence of flavonoids and saponins in aqueous and ethanolic extracts may explain their powerful wound healing activity. The flavonoid components present in the seed suppress lipid peroxidation, improving the viability of collagen fibers, while saponins aid wound healing by increasing the rate of keratinocyte migration. Saponins also decreasethe number of inflammatory cells in wounds (Georgescu et al., 2016). Epithelialization is crucial because it preserves the skin's integrity, rendering it less prone to infections (Pastar et al., 2014). The estimated epithelization day for all of the treatments, along with the control and normal drug, was 17.4 days, while the average epithelization day for the aqueous, ethanol extracts was 16.13 days. The epithelization time of the aqueous and ethanol extracts was almost identical to the epithelization time of silver sulfadiazine ointment.

Thus, on the basis of the results it can be conveniently inferred that the aqueous and ethanolic extracts have the ability to cure wounds in line with the commercially available wound treating medicines.

Incision wound model

Increased collagen concentration and fiber stability may reflect the higher tensile strength (Shoulders, 2009). Table 9 shows the wound healing activity data of the extracts analysed using an incision wound model. Incised tissue tensile strength for groups I, II, III and IV was found to be 228.2, 312.6, 275.4 and 283.5 g/mm^2, respectively. The tensile strengths of the aqueous (20.67%) and ethanolic extracts (24.23%) extracts were found to be equal to that of the silver sulfadiazine ointment. In terms of tensile strength, the ethanolic extract ointment showed good wound healing activity, while the petroleum ether extract showed fair wound healing activity. From this study, it can be said that seed extracts of *B. juncea* not only improved collagen synthesis, but also assisted in protein cross-linking.

Table 9 shows the weights of wet and dry granulation tissue. Normal saline, silver sulfadiazine, aqueous and ethanolic extract ointment treated animals had wet and dry granulation weights of 212.4 & 67.8, 346.7 & 121.2, 331.3 & 107.4, 340.1 & 111.2 g, respectively. The wound healing ability of *B. juncea* aqueous and ethanolextracts was widely demonstrated, as evidenced by the research findings in the incision wound model, verifying the statement experimentally as indicated in traditional medicine.

Conclusion

It can be concluded from the findings of the present study that the seed extracts of *B. juncea* contain bioactive compounds exhibiting primarily anti-inflammatory and wound healing activities. Thus the seeds can be seen as a lead in the quest for new chemical substances capable of anti-inflammatory and wound-healing properties as well as for the production of appropriate formulation dosage forms to treat inflammation and burn-inflicted wounds.

References

[1] Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC

Complement Altern Med., 2015 ;15:159.

[2] Agar OT, Dikmen M, Ozturk N, Yilmaz MA, Temel H, Turkmenoglu FP. Comparative Studies on Phenolic Composition, Antioxidant, Wound Healing and Cytotoxic Activities of Selected Achillea L. Species Growing in Turkey. Molecules, 2015;20(10):17976-8000.

[3] Alam F, Us Saqib QN. Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of *Zanthoxylum armatum* (Rutaceae). Anc Sci Life, 2015; 34(3):147-55.

[4] The Ayurvedic Pharmacopoeia of India (2001). 1st Edition, Part I, Volume I. Delhi (IN): Government of India, Ministry of Health and Family Welfare, Department of Indian Systems of Medicine & Homoeopathy.

[5] Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound healing dressings and drug delivery systems: a review. J Pharmaceu Sci, 2008; 97(8):2892–2923.

[6] Builders PF, Kabele-Toge B, Builders M, Chindo BA, Anwunobi PA, Isimi YC. Wound healing potential of formulated extract from hibiscus sabdariffa calyx. Indian J Pharm Sci, 2013; 75(1):45-52.

[7] Bunman S, Dumavibhat N, Chatthanawaree W, ntalapaporn S, Thuwachaosuan T, Thongchuan C. Burn Wound Healing: Pathophysiology and Current Management of Burn Injury. The Bangkok Medical Journal, 2017; 13:91–8.

[8] Chandra S, Khan S, Avula B, Lata H, Yang MH, Elsohly MA, Khan IA. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. Evid Based Complement Alternat Med, 2014:253875.

[9] Chen LG, Yang LL, Wang CC. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. Food Chem Toxicol, 2007.

[10] Chuong CM, Nickoloff BJ, Elias PM, Goldsmith LA, Macher E, Maderson PA, Sundberg JP, Tagami H, Plonka PM, Thestrup-Pederson K, Bernard BA, Schröder JM, Dotto P, Chang CM, Williams ML, Feingold KR, King LE, Kligman AM, Rees JL, Christophers E. What is the 'true' function of skin?. Exp Dermatol, 2002; 11(2):159- 87.

[11] Dinda SC, Mukharjee B. Gum cordia – A new tablet binder and emulsifier. Acta Pharmaceutica Sciencia, 2009; 51:189-198.

[12] Doss A. Preliminary phytochemical screening of some Indian Medicinal Plants. Anc Sci Life,2009; 29(2):12-6.

[13] Działo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A. The Potential of Plant Phenolics in Prevention and Therapy of Skin Disorders. Int J Mol Sci., 2016; 17(2):160.

[14] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol, 2014; 4:177.

[15] Evans WC. Quality Control. In: Trease and Evans Pharmacognosy. 15th edn. Sounder's Company Ltd.; New York: 2004:9-105.

[16] Georgescu M, Marinas O, Popa M, Stan T, Lazar V, Bertesteanu SV, et al. Natural Compounds for Wound Healing. In: Worldwide Wound Healing - Innovation in Natural and Conventional Methods, 2016.

[17] Khandayataray P, Murthy MK. Qualitative and Quantitative Phytochemical Screening, Antioxidant and Anti-inflammatory Activities of Acetone Extract of *Brassica juncea* L. Leaf. AJRB 2019:1–15.

[18] Khandelwal KR. Evaluation of crude drugs. In: Pharmacognosy Thechniques and Experiments. 19th edn. Nirali Prakashan; Pune: 2008:157-160.

[19] Kokate CK. Evaluation of crude drugs. In: Practical Pharmacognosy. 4th edn. Vallabh Prakashan; New Delhi: 2007:122-125.

[20] Kokate CK, Purohit AP, Gokhale SB. Analytical Pharmacognosy. In: Pharmacognosy. 38th edn. Nirali Prakashan; Pune: 2007:109-114.

[21] Le B, Anh P, Yang S. Enhancement of the Anti-Inflammatory Effect of Mustard Kimchi on RAW 264.7 Macrophages by the Lactobacillus plantarum Fermentation-Mediated Generation of Phenolic Compound Derivatives. Foods, 2020; 9:181.

[22] Mathur R. Evaluation of Pharmacological activities of Herbal Drugs. In: Drug Screening Methods (Preclinical Evaluation of new Drugs), Gupta SK (editor). 2nd edn. Jaypee Brothers Medical Publishers (P) Ltd.; New Delhi, India: 2009:237-242.

[23] Mechesso AF, Tadese A, Tesfaye R, Tamiru W, Eguale T. Experimental evaluation of wound healing activity of croton macrostachyus in rat. African J Pharm Pharmacol, 2016; 10(39):832–838.

[24] Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. Clinics in Dermatology, 2007; 25(1):19–25.

[25] Mujumdar AM, Naik DG, Dandge CN, Puntambekar HM. Antiinflammatory activity of Curcuma amada Roxb. in albino rats. Indian J Pharmacol, 2000; 32:375-377.

[26] Muscará MN, McKnight W, Asfaha S, Wallace JL. Wound collagen deposition in rats: effects of an NO-NSAID and a selective COX-2 inhibitor. British Journal of Pharmacology, 2000; 129(4):681–686.

[27] OECD, OECD guide lines for the testing of chemicals/ section 4: Health effects test no 423: Acute oral toxicity – Acute toxic class method. OECD; Paris: 2002.

[28] Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle- income countries: a WHO-SAGE study. Health Policy Plan, 2016; 31(8):984-91.

[29] Parmer NS, Prakash S. Evaluation of analgesics, anti-

inflammatory and antipyretic activity. In: Screening Methods in Pharmacology. 1st edn. Narosa Publishing House Pvt. Ltd.; New Delhi, India: 2006:211-215.

[30] Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Patel SB, Khalid L, Isseroff RR, Tomic-Canic M. Epithelialization in Wound Healing: A Comprehensive Review. Adv Wound Care (New Rochelle), 2014; 3(7):445-464.

[31] Patil S, Anarthe S, Jadhav R, Surana S. Evaluation of anti-inflammatory and In-vitro Antioxidant Activity of Indian Mistletoe, the Hemiparasite Dendrophthoe falcata L. F. (Loranthaceae). Iranian J Pharm Res, 2011; 10(2):253-259.

[32] Peden M, Oyegbite K, Ozanne-Smith J, et al., editors. World Report on Child Injury Prevention. Geneva: World Health Organization; Burns, 2008; 4.

[33] Rahman M, Khatun A, Liu L, Barkla BJ. Brassicaceae Mustards: Traditional and Agronomic Uses in Australia and New Zealand. Molecules, 2018; 23(1):231.

[34] Rathi BS, Bodhankar Baheti AM. Evaluation of aqueous leaves extract of Moringa oleifera Linn for wound healing in albino rats. Ind J Exp Bio, 2006; 44:898-901.

[35] Sankhalkar S, Vernekar V. Quantitative and Qualitative Analysis of Phenolic and Flavonoid Content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L. Pharmacognosy Res, 2016; 8(1):16-21.

[36] Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC, Longaker MT. Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen, 2009; 17(6):763-71.

[37] Shoulders MD, Raines RT. Collagen structure and stability. Annu Rev Biochem., 2009; 78:929-58.

[38] Singh O, Gupta SS, Soni M, Moses S, Shukla S, Mathur RK. Collagen dressing versus conventional dressings in burn and chronic wounds: a retrospective study. J Cutan Aesthet Surg, 2011; 4(1):12-6.

[39] Somboonwang J, Kankaisre M, Tantisira B, Tantisira MH. Wound healing activities ofdifferent extracts of *Centella asiatica* in incision and burn wound models: an experimental animal study. BMC Complem Altern Med, 2012; 12:103.

[40] Tian Y, Deng F. Phytochemistry and biological activity of mustard (*Brassica juncea*): a review. CyTA - J Food, 2020; 18:704-18

[41] Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects:An Overview. Medicines (Basel), 2018; 5(3):93.

[42] Tunner RA. Acute Toxicity studies. In: Screening methods in Pharmacology. Vol. 1. 4th edn. Academic Press, an Imprint of Elsevier; Haryana, India: 2009:302-303.

[43] Vafi F, Bahramsoltani R, Abdollahi M, Manayi A, Hossein Abdolghaffari A, Samadi N, Amin G, Hassanzadeh G, Jamalifar H, Baeeri M, Heidari M, Khanavi M. BurnWound Healing Activity of *Lythrum salicaria* L. and *Hypericum scabrum* L. Wounds, 2016:WNDS20160929-2.

[44] Venkataswamy R, Doss A, Sukumar M, Mubarack HM. Preliminary Phytochemical Screening and Antimicrobial Studies of *Lantana indica* Roxb. Indian J Pharm Sci, 2010; 72(2):229-31.

[45] Vijayaram S, Kannan S, Saravanan KM, Vasantharaj S, Sathiyavimal S, P PS. Preliminary phytochemical screening, Antibacterial potential and GC-MS analysis of two medicinal plant extracts. Pak J Pharm Sci, 2016; 29(3):819-22.

[46] Ye H, De S. Thermal injury of skin and subcutaneous tissues: A review of experimental approaches and numerical models. Burns, 2017; 43(5):909-932.