ISSN 2581 – 4303

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2018; 9(2):91-100

RESEARCH ARTICLE

Development, Characterization, and Isolation of Alkaloidal Fraction from *Tephrosia purpurea* and Evaluate its Wound Healing Activity

Gaurav Dubey[1], Anand Chaurasiya[2], Ravindra Pal Singh[3]

[1]*Department of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India,* [2]*Department of Pharmacy, Millennium College of Pharmacy, Bhopal, Madhya Pradesh, India,* [3]*Department of Pharmacy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India*

Received: 02 May 2018; Revised: 25 May 2018; Accepted: 05 Jun 2018

ABSTRACT

A wound is a break in the skin. Wound is usually caused by cuts or scalps, and symptoms at wound or injury include swelling, stiffness, tenderness, discoloration skin tightness, itching, and scar formation, two types of tissue injury. Wound healing is a complex dynamic process. The main objective of this investigation is to study the development, characterization, and isolation of alkaloidal fraction from *Tephrosia purpurea* and evaluate its wound healing activity in various wound models such as excision, incision, dead space, and burn wound models. Various evaluation parameters such as wound contraction, epithelization time, tensile strength, wet and dry granuloma weight, and hydroxyproline estimation were performed. The main objective of this investigation is to develop a product, which may give a wound healing property, and enhance wound healing process such as increase the collagen synthesis, fibroblast proliferation, angiogenesis, and epithelization because products which are available in market are either antiseptic or antimicrobial.

Keywords: Scar, swelling, tenderness, wound.

INTRODUCTION

The skin is the most important organ of the body which covers a total area of about 20^2 feet. The skin protects the human body from microbes and the elements and helps to regulate body temperature.[1] A wound is basically a break in the skin. Any Wound are usually caused by cuts and scalps and various symptoms associated with a wound are swelling, stiffness, tenderness, change the colour of skin. Skin tightness, scabbing, itching and scar formation tissue.[2] Flavonoids are low molecular weight bioactive polyphones which play an important role in photosynthesis. Flavonoids showing various pharmacological activities such as anti-inflammatory, antibacterial, antiviral, anti-allergic antitumor, treatment of neurodegenerative diseases, and wound healing activity of selected plants were performed using various wound healing models such as incision, excision, dead space, and burn wound model.[3] *Tephrosia purpurea* commonly known as *Sharpunkha* belongs to Fabaceae family. The present study was aimed to study the development, characterization, and isolation of alkaloidal fraction from *T. purpurea* and evaluates its wound healing activity using various wound healing models.[4]

***Corresponding Author:**
Gaurav Dubey
Email: gaurav1980dubey@gmail.com

MATERIALS AND METHODS

Collection and authentication of plant material

Aerial parts of *T. purpurea* were collected during the June–August and late September from the surrounding area of Bhopal (MP). The aerial parts and leaves collected were shade dried using a tray under the controlled temperature at 35°C. Plants were then drug converted into powder by the help of pulverize, and the powder was stored in polythene bags which free from microbes. The plant material was identified from the Department of Botany, SAFIA College, Bhopal (Voucher No. 281/bot.1/SAF/12) and a specimen deposit in the institute. The crude drug was then shade dry and crushed in small pieces for extraction and extractive values.

Determination of physical parameters[5]

The physical specification is to be determined, and these are rarely constant for each crude drugs but may help in evaluation, specifically for moisture content, foreign organic matter, ash value, and extractive value.

Qualitative analysis of extracts[5-7]

The primary tests for the detection of various metabolites were carried out on selected plants by adopting standard procedures. The extracts obtained by selected extraction method were used to perform the identification test for the presence of alkaloids, glycosides, carbohydrates, phytosterols, saponins, tannins, phenolic compound proteins and free amino acids, and flavonoids.

Purification by chromatographic techniques

Chromatography is a laboratory technique used for the separation of individual compounds from molecular mixtures. Chromatography may be preparative or analytical. Preparative chromatography is basically used to separate the individual components of a mixture for more advanced use. Meanwhile, the analytical chromatography is used for measuring the relative proportions of analytes in a mixture. The extraction procedures yield a variety of components. These components may have the same groups of compounds or the extract may contain a more diverse mixture in which only certain type of the constituents is biologically active. It is unusual to obtain an extract which is crystallizable or which can be readily and completely standardized with regard to its activity. The final product of an extraction procedure usually consists of a mixture of related compounds, and their resolution requires the basic understanding of their physical and chemical characteristics.

Separation of alkaloids

Extraction

100 g plant material wetted with 50 ml of half diluted aqueous NH_4OH and lixiviated overnight with 1000 ml EtOAc. The filtered organic solution was extracted with 2% H_2SO_4. The resulted organic phase was separated using separating funnel and basified with NH_4OH. This is extracted with 1000 ml of $CHCl_3$ and was dried over Na_2SO_4 and evaporated to dryness at 40°C in vacuum

Development

20 µl of redissolved alkaloid extracts were loaded with a capillary tube on a percolated Alugram®Sil G/F_{254} plate and was allowed to dry. 20 µl of brucine was also loaded as a standard alkaloid. The plate was kept in a saturated chromatographic chamber containing the mixture of chloroform and methanol (15:1) as a mobile solvent phase.

Detection

The developed chromatographic plates were observed first under short wavelength ultraviolet light ($UV_{250\ nm}$) chamber and recorded the color and hRf values of bands.

Separation of active fraction from selected plant using column chromatography[8]

This technique is based on the differential adsorption of the substrate by the adsorbent. The column in which the stationary phase was packed consisted of a glass tube, typically 3.5 cm in diameter and 80–100 cm in length. The bottom of the column was fitted with a stopcock which provides control on the flow rate of mobile phase. The packing material was supported inside the column by inbuilt sintered glass. It was thoroughly cleaned, dried, and checked for any type of leakage. The isolated single compound was subjected to the following physical and chemical tests to use these data as the basis in the process identification and structure identification of compounds such as nature color, hRf values, melting point, solubility, and yield. The purified compounds were identify by UV, Fourier transform infrared, 1H Nuclear magnetic resonance (NMR), and 13 NMR mass spectroscopic studies and obtained the special data, which is of immense use in the detection of the functional groups and further to elucidate their structure.

Formulation of ointment

Ointments of *T. purpurea* and its isolated alkaloidal fraction were prepared using simple ointment base BP. All the doses for the test extract were

fixed from the acute toxicity studies.[9] Topical application of extract was used in excision, incision, and burn wound model and dead space wound model receive oral suspension. 5% w/w of extract ointments was used during the study [Table 1].[10]

Formulation of suspension

Suspensions of *T. purpurea* and its isolated alkaloidal fraction were prepared using the following formula. Suspension of test drug extracts was prepared by mixing 2 g of drug with 20 ml of tragacanth mucilage. Mucilage was prepared using the formula given below, in which purified water added to make it 100 g.

Procedure

Glycerin 18gm, water 75ml were mixed in a tarred vessel and heated. The mixture was heated till boiling point and then adds Tragacanth 6gm and Benzoic acid 0.2gm, then added enough purified water, stirred actively until they form uniform consistency and strained forcibly through muslin. [Table 2].

Wound healing activity

Selection of animals

After taking approval from the Institutional Animals Ethics Committee (TIT/IAEC/831/P'COLOGY/2015/54), rats of Wistar strain were selected. They maintained at 26–30°C, housed independently. The animals were left free for 48 h to maintain them in the normal room conditions. Standard pellet diet was given to them. To perform the experiment, the rats were divided into four groups (n = 6).[11] The results were analyzed by one-way ANOVA and a $P < 0.01$ was considered statistically significant.

Table 1: Composition of simple ointment base for control group (100 g)

Constituents	Quantity
Polyethylene glycol 400	40 g
Polyethylene glycol 600	60 g

Table 2: Composition of tragacanth mucilage (100 g)

Constituents	Quantity
Glycerin	18 g
Purified water	75 ml
Tragacanth	2 g
Benzoic acid	0.2 g

Selection of model

Excision, incision, dead space, and burn wound model using Wistar albino rats was selected for performing the wound healing activity. The various parameters for the evaluation of wound healing activity are rate of wound contraction, time required for full epithelization, tensile strength, granuloma weight, and hydroxyproline estimation. These parameters were successful in evaluation because of easy availability of albino rat and simplicity in handling them.

Excision wound model[12]

In this particular model, Wistar rats were selected and their hairs were removed from dorsal thoracic region before wounds were created. Diethyl ether used was used as an anesthetic agent. A wound of about 2.5 cm diameter was made which was circular on dorsal thoracic region of rats under aseptic conditions and was observed throughout the study. Immediately, the areas of the wounds were measured (in mm^2) by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it (approximate area 500 mm^2). The animal of Group I treated as control and only ointment base applied topically. The animal of Group II treated as Test I which received ointment o*f T. purpure*a, animal of Group III treated as Test II which received ointment of alkaloidal fraction of *T. purpure*a topically, and Group IV contains standard drug which received povidone-iodine daily for 16 days, starting from the day of wounding. The observations of percentage wound closure were made on the 4^{th}, 8^{th}, 12^{th}, and 16^{th} post-wounding days. The wound area of each animal was measured using tracing paper method. The percentage of wound contraction was calculated from the days of measurements of wound area.

Incision wound model[12]

In this particular wound model, Wister rats were selected and they shave by removing hairs at the dorsal thoracic region. Diethyl ether was used as a anesthetic agent. 6 cm long paravertebral incisions were made through the full thickness of skin on

either side of vertebral column of the rat. The wounds were closed with interrupted sutures of 1 cm apart. The rats are categorized into four groups (n = 6). The animal of Group I treated as control and only ointment base applied topically. The animal of Group II treated as Test I which received ointment of *T. purpurea*, animal of Group III treated as Test II which received ointment of alkaloidal fraction of *T. purpurea* topically, and Group IV contains standard drug which received povidone-iodine daily for 16 days, starting from the day of wounding. All the samples were applied once daily for 16 days. The sutures were removed on 8th post-wounding day. The tensile strength of wounds was measured on the 10th day following continuous water flow technique.

Dead space wound model[12]

In this particular wound model, a grass pith (2.5 cm × 0.3 cm) is selected and sterilized after that this grass pith implants in a dead space wound model using light ether anesthesia on either side of the dorsal paravertebral surface of rat. The animal of Group I treated as control and only suspension base given orally. The animal of Group II treated as Test I which received a suspension of *T. purpurea*, animal of group III treated as Test II which received suspension of alkaloidal Fraction of *T. purpurea* topically, and Group IV contains standard drug which received povidone-iodine daily for 16 days, starting from the day of wounding. On the 10th day of wounding, granuloma tissue which was formed on grass p pith was excised. The weight of wet and dry granulation tissues was measured along with an estimation of biochemical parameter like hydroxyproline estimated.

Dry granuloma weight

The granuloma was collected from grass pith at the 10th day from dead space wound model. The granuloma was dried at 6°C for 3 h and weighted.

Hydroxyproline estimation in dead space model

The granuloma tissue was collected for the estimation of hydroxyproline. Calculated quantities of tissue sample were immersed in 2 mL of 6 M-HCl, and the tubes were sealed without evacuation. Hydrolysis was done for 3 h at 105°C. After hydrolysis of tissue, 50 µl of sample was taken and 0.4 mL isopropanol was mixed to it. Then, 0.2 mL of solution A was mixed and incubated at room temperature for 5 min. After incubation, 2.5 mL of solution B was mixed and incubated at 58°C for 25 min. Then, this mixture was cooled in tap water and absorbance was taken at 558 nm within 30 min. The quantity of hydroxyproline was calculated with the help of standard curve.

Burn wound model[13]

Albino rats of Wistar strain (150–200 g) body weight were selected and maintained at uniform temperature and diet in well-ventilated cages. Medium thick burn wound was inflicted on overnight starved animal under light ether anesthesia using a metal rod (1.5 cm in diameter) heated to 80–850°C and exposed for 20 s after 24 h dead tissue was excised using sterile surgical blade. Wound contraction was measured after completion of the model.

RESULT AND DISCUSSION

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant such as bark, leaves, flowers, roots, fruits, and seeds. Knowledge of the chemical constituents of plants is desirable for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers. On the basis of ethnobotanical studies, detailed literature survey on aerial parts of *T. purpurea* was selected because it contains various constituents which enhance wound healing activity as per our literature survey. Ash value determination is a very important tool to access the quality of herbal raw material since higher ash value is an indication of adulteration and/or improper processing of raw material. Results for the percentages of total ash, acid-insoluble ash, and water-soluble ash are shown in Table 3. Moisture is an unavoidable component of the crude drugs, and it must be reduced as much as possible. Drying of the crude drug will help in their preservation, and the results are shown in Table 3. Herbal drugs should be devoid of insects, molds, animal excreta, and other contaminants such as soil, stone, dust, and other extraneous

matter such as glass pieces and metal parts. The results are shown in Table 3.

Extractive value determinations tell us the amount of phytoconstituents which is present in the medicinal plant. Under a given set of conditions, these values vary within a narrow limit and hence can be set as an in-house standard for routinely used drugs. These values can also tell us about the adulteration of crude drug with already exhausted drug as it will yield low extractive values. The results of the extractive values are shown in Table 4.

The selection of solvent depends on the fact that how much it dissolved the required phytoconstituents. Aerial part of *T. purpurea* was extracted using 90% ethanol [Table 5].

Plants are known to contain various primary metabolites such as sugar and fats which are used by animals and humans. They also contain many secondary metabolites which show certain physiological effects. Qualitative phytochemical test was perform on all three selected plants which shown the presence of various metabolites. All the extracts of selected medicinal plant were undergone for chemical test, and the results are shown in Table 6.

Table 3: Physicochemical parameters

Parameters	*T. purpurea* (%)
Moisture content	8.65
Total ash	7.15
Acid-insoluble ash	0.93
Water-soluble ash	2.58
Foreign organic matter	0.31

T. purpurea: Tephrosia purpurea

Table 4: Extractive values

Extractive values	*T. purpurea* (%)
Alcohol soluble	13.89
Water soluble	19.1

T. purpurea: Tephrosia purpurea

Table 5: Extraction procedure

Plant name	Part used	Solvent used	Procedure
T. purpurea	Aerial part	90% ethanol	Soxhlet extraction

T. purpurea: Tephrosia purpurea

Table 6: Qualitative phytochemical test

Serum metabolites	Ethanol extract
Alkaloids	+
Phenols	+
Flavonoids	+
Tannins	+
Triterpenoids	+
Steroids	+
Saponins	+
Glycosides	+

Qualitative separation of *T. purpurea* by thin-layer chromatography

The thin-layer chromatography of *T. purpurea* was performed using chloroform and ethyl acetate (8:2) as a developing solvent and sulfuric acid as a spraying reagent. The chromatogram of ethanol extract of *T. purpurea* in the solvent system chloroform:ethyl acetate (60:40) displayed four distinct bands possessing dark blue, dark blue, yellow, yellow blue with hRf values 54, 57, 68, and 83, respectively. The chromatogram of ethanol extract of *T. purpurea* in the solvent system chloroform:ethyl acetate (60:40) displayed six distinct bands possessing various color with hRf values 8, 10, 12, 16, 18, and 20, respectively. The chromatogram of ethanol extract of *T. purpurea* in the solvent system chloroform:ethyl acetate (60:40) displayed three distinct bands possessing green, blue, and light green with hRf values 60, 68, and 78, respectively [Table 7].

Table 7: Qualitative separation from *Tephrosia purpurea*

Secondary metabolites	Number of bands	hRf values	Color of the bands
Flavonoids	4	54	Dark blue
		57	Dark blue
		68	Yellow
		83	Yellow blue
Phenols	6	8	Blue
		10	Blue
		12	Dark blue
		16	Dark blue
		18	Blue
		20	Blue
Alkaloids	3	60	Green
		68	Blue
		78	Light green

Separation of alkaloid fractions from *T. purpurea* by column chromatography

It is evident from the earlier studies and literature that *T. purpurea* is a rich source of alkaloids, phenols, and flavonoids of pharmacological importance. Thus, an attempt was made to isolate some of these alkaloid fractions from *T. purpurea* by column chromatography. The

ethanol extract of *T. purpurea* was filled in Silica gel-H column at 26°C and lbr pressure. Removal of 200 ml dead volume we obtain 20 fractions of 100 ml. 1 to 11 was obtained from the petroleum ether:chloroform, and 12–20 was collected from chloroform:ethyl acetate. A total of 20 fractions were collected, and the concentrated solutions of these fractions had waxy nature. Fractions, 1–11, were waxy and 12–20 were semi-solid [Table 8]. Column chromatography, using petroleum ether chloroform-ethyl acetate-methanol as mobile phase, yielded one major alkaloidal compound 2-(3-hydroxyphenyl)-3-methoxy-4*H*-furo[2,3-*h*] chromen-8(9*H*)-on [Figure 1].

Wound healing activity

Selection and procurement of animals

After taking permission for animal studies from the Institutional Animal Ethical Committee (TIT/IAEC/831/P'COLOGY/2015/54), Wistar albino rats were procured and male rats weighing 150–200 g were selected, maintained at 24–28°C, and housed individually with free access to food and water. The animals were left for 48 h to acclimatize to the animal room conditions. They were fed with standard diet. For the evaluation of wound healing activity, four groups were prepared for incision, excision, and burn wound model which shows in and divided in control, Test I, Test II, and standard drug, and for dead space wound model, four groups were prepared which divided into control, Test I, Test II, and standard drug.

Wound contraction and epithelization time in excision wound model

The wound contraction was calculated as the percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required

Figure 1: Separation of alkaloids by column chromatography

Table 8: Isolation of compound fractions through column chromatography

M phase	Ratio	Number of fraction	Color	Nature of extract
Petroleum ether: chloroform	100:00	1	Yellow	Waxy
Petroleum ether: chloroform	90:10	1	Light Yellow	Waxy
Petroleum ether: chloroform	80:20	1	Greenish Yellow	Waxy
Petroleum ether: chloroform	70:30	1	Light Green	Waxy
Petroleum ether: chloroform	60:40	1	Green	Waxy
Petroleum ether: chloroform	50:50	1	Dark Green	Waxy
Petroleum ether: chloroform	40:60	1	Dark Green	Waxy
Petroleum ether: chloroform	30:70	1	Light Brown	Waxy
Petroleum ether: chloroform	20:80	1	Light Brown	Waxy
Petroleum ether: chloroform	10:90	1	Greenish Brown	Waxy
Petroleum ether: chloroform	00:100	1	Greenish Brown	Waxy
Chloroform: ethyl acetate	100:00	2	Light Brown	Semi Solid
Chloroform: ethyl acetate	90:10	2	Brown	Semi Solid
Chloroform: ethyl acetate	80:20	2	Dark Brown	Semi Solid
Chloroform: ethyl acetate	70:30	2	Orange Red	Semi Solid
Chloroform: ethyl acetate	60:40	2	Green	Semi Solid
Chloroform: ethyl acetate	50:50	2	Blue	Semi Solid
Chloroform: ethyl acetate	40:60	2	Light Green	Semi Solid
Chloroform: ethyl acetate	30:70	2	Yellow	Semi Solid
Chloroform: ethyl acetate	20:80	2	Light Yellow	Semi Solid

for scar to fall off leaving no raw wound behind. Effect of control, Test I, Test II, and standard drug (povidone-iodine) was observed on percentage wound contraction in excision wound model on initial, 4th, 8th, 12th, and 16th days interval which is shown in Table 9. It has been seen that significant wound healing took place in case of animals treated with ointment of *T. purpurea* which is 19 days and ointment of alkaloidal fraction of extract of *T. purpurea* took 20 days for complete wound healing. The least rate of wound healing was seen in control group which received no treatment, and the fastest rate of wound healing was seen in standard drug group where animals received standard drug which is povidone-iodine, and wound contraction is shown in Figure 2. Epithelization period (days) in excision wound healing model is also shown in Graph 1.

Measurement tensile strength

The tensile strength was calculated, on 10th day, animal were anesthetized and kept them on the board, and the effect of control, Test I, Test II, and standard drug was observed which is shown in Table 10 which indicates that animals treated with isolate of *T. purpurea* highest tensile strength after that Test I-treated animals shown their tensile strength. The minimum tensile strength was seen in control where animal not received any treatment and maximum tensile strength was seen in standard drug group where animals received standard drug which is shown in Graph 2. Measurement of tensile strength was done by the tensiometer.

Wet granuloma, dry granuloma weight, and hydroxyproline measurement for dead space wound model

Effect of control, Test I, Test II, and standard was observed on dry, wet granuloma weight and proline estimation which is shown in Table 11 which indicates that animals received suspension of *T. purpure*a shows highest weight of wet and dry granules and proline (mg/g of tissue) Estimation after that isolate of *T. purpure*a treated animals shows their wet and dry granuloma weight, proline Estimation, control group where animals not received any treatment shows least and standard drug received animale shows highest

Table 9: Shrinkage of wound area (%)

Class	Area of wound closure (mm²±SEM)					
	Initial	4th	8th	12th	16th	Complete recovery
I (Control)	10.82±0.68	18.82±0.68	38.12±1.80	48.21±1.80	68.69±2.60	24
II (Test I)	14.14±0.98*	35.14±0.54*	52.14±0.85*	75.12±0.79*	93.99±0.68*	19
III (Isolate T.P)	15.19±1.25	30.16±0.75*	45.29±1.95*	60.62±0.95*	80.16±1.65*	20
IV (Standard)	15.26±1.25*	42.24±1.07*	65.34±1.70*	90.12±1.08*	100±0.75*	16

#Initial area. 500 mm². ≈*n*=6 rats in each class. ≠Result shown as mean area±SEM. SEM: Standard error mean

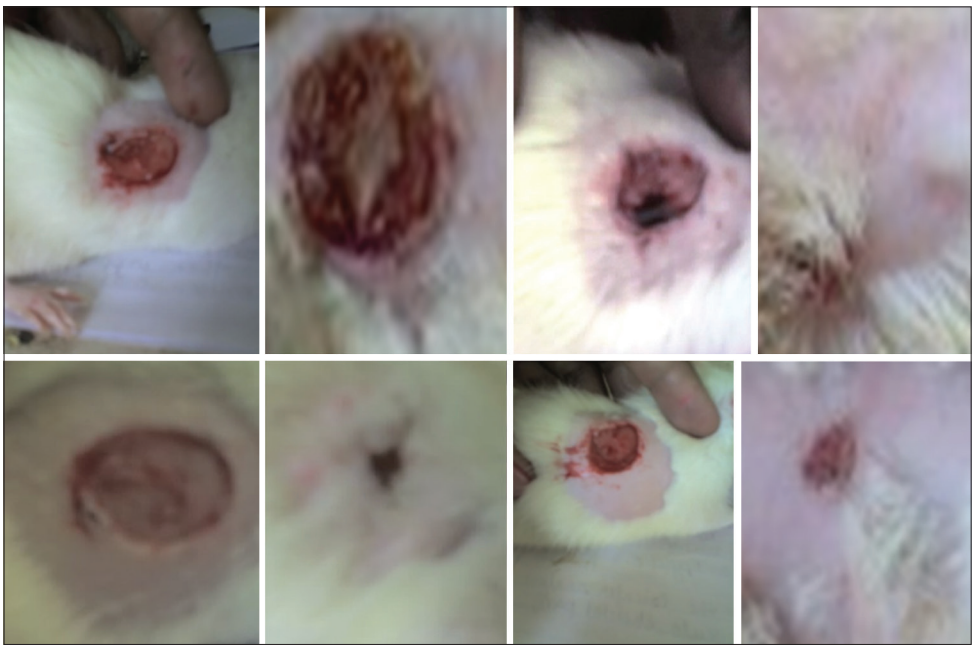
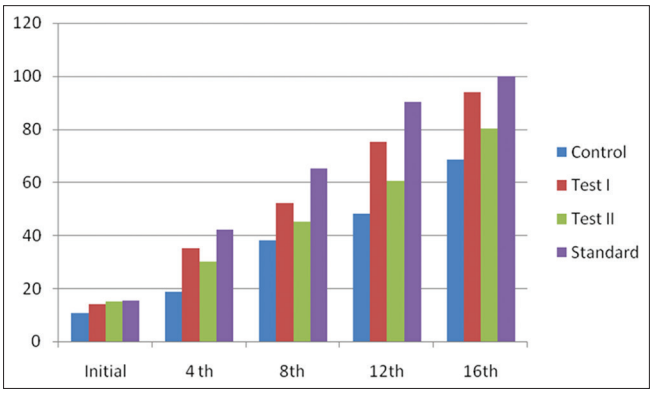


Figure 2: Wound contraction in excision wound model (1st and 16th days)

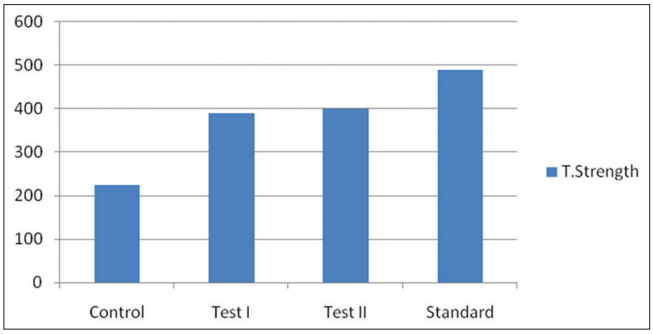
Table 10: Tensile strength in incision wound model

Groups	Tensile strength (in g)
Control	225.16±3.51
Test I	390.29±2.56
Isolate of Test I (T.P)	399.92±1.56
Standard	490.50±2.71

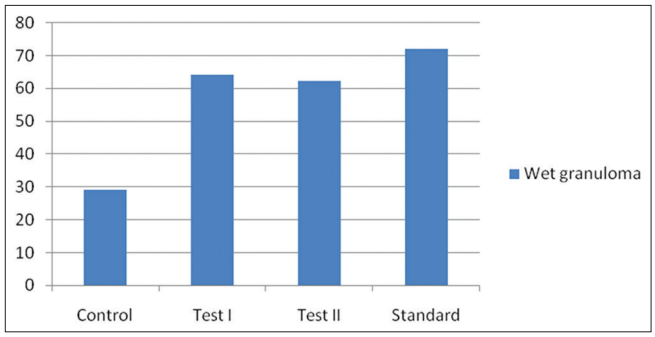
≠Result expressed as mean area±SEM. *$P \leq 0.01$ means importance when compared to control. SEM: Standard error mean



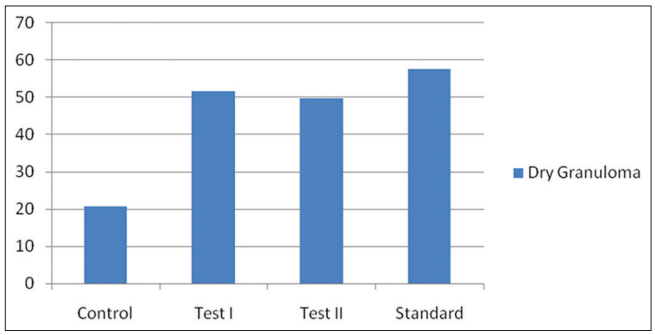
Graph 1: Epithelization period (days) in excision wound healing model



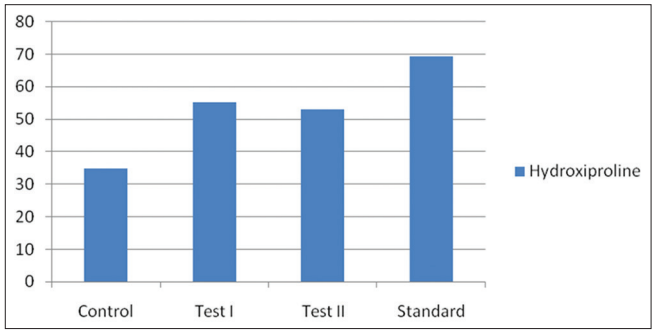
Graph 2: Tensile strength (g) in incision wound healing model



Graph 3: Effect of extract on wet granuloma weight



Graph 4: Effect of extract on dry granuloma weight



Graph 5: Effect of extract on hydroxiproline estimation

wet, dry granuloma weight and proline estimation. Graphs 3-5 indicate the effect of extract on wet, dry granuloma weight, and proline estimation.

Wound contraction and epithelization time in burn wound model

Effect of control, Test I, Test II, and standard drug was observed on percentage wound contraction in burn wound model on initial, 4th, 8th, 12th, and 16th days interval which is shown in Table 12 which indicates that highest wound contraction took place in case of animals treated with ointment of *T. purpurea* which took 19 days for healing. After that, isolate of *T. purpurea* took 20 days for complete healing. The least recovery was seen in control and took 24 days for complete healing, and fastest recovery was shown by standard drug which is silver sulphadiazine which took 16 days for complete healing. Percentage wound contraction in burn wound model is shown in Graphs 6 [Figure 3].

CONCLUSION

The skin is a very important organ of human body which covers approximately 20^2 feet of human body. The skin provides defensive mechanism for the body against various microbes, infection, and elements. It regulates body temperature. Alkaloids show various biological activities such as anti-inflammatory, antibacterial, antiviral, anti-allergic, antitumor, treatment of neurodegenerative diseases, and vasodilatory action. Flavonoids are inhibiting lipid-peroxidation platelet aggregation, capillary permeability and fragility, cyclooxygenase, and

Table 11: Dry and wet granuloma weight and proline estimation

Group (n)	Wet granuloma	Dry granuloma	Hydroxyproline (mg/g of tissue)
Control	29.12±1.20	20.71±3.20	34.70±4.92
Test I	64.19±2.12	51.66±1.23	55.12±3.23
Isolate of Test I (T.P)	62.22±2.16	49.66±1.13	53.12±3.63
Standard	72.19±1.66	57.62±2.12	69.35±3.22

≠Result expressed as mean area±SEM. *$P \leq 0.01$ shows importance when compared with control. SEM: Standard error mean

Table 12: Percentage wound contraction in Burn wound model

Class	Shrinkage of wound in square millimeter					
	Initial	4th	8th	12th	16th	Complete recovery
I (Control)	5.92±0.72	20.19±0.92	40.22±1.80	60.11±1.21	70.19±1.20	24
II (Test-I)	13.11±0.92	38.92±1.21	55.12±0.92	74.12±0.86	84.99±0.72	19
III (Isolate of T.P)	14.11±0.92	40.92±1.01	52.12±1.92	70.12±0.86	88.99±0.72	20
IV (silver sulfadiazaine)	12.13±2.12	40.24±1.24	66.12±1.29	91.92±0.92	99.19±0.71	16

#Initial wound area approx. 500 mm2. ≈n=6 rats/class. ≠Result shown as mean area ± SEM*. $P \leq 0.01$ shows importance when compared to control. SEM: Standard error mean

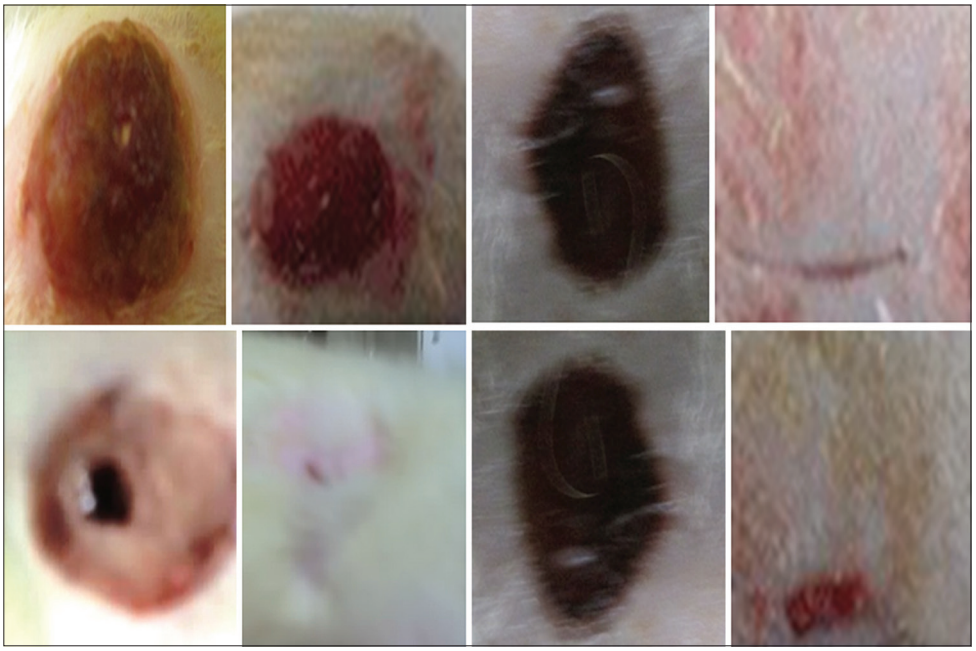
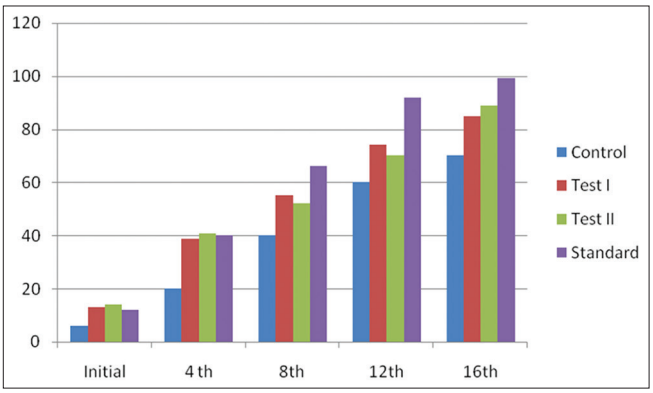


Figure 3: Wound contraction in Burn wound model (1st and 16th days)



Graph 6: Shrinkage of wound

lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation, and these are also reported to inhibit a variety of enzymes such as hydrolases, hyaluronidase, alkaline phosphatae, arylsulfatase, CAMP phosphor diesterase, lipase, α-glucosidase, and kinase (Bimlesh *et al.* 2011). Alkaloids enhance wound healing activity which proves by various researches. Based on the present investigation, it was well understood that alkaloidal fraction of *T. purpurea* shows significant wound healing activity in all selected wound healing models as compared to standard drug. It was also found that *T. purpurea* extract and its alkaloidal fraction show increasing wound contraction and epithelization time, increasing tensile strength, and increasing wet and dry granuloma weight.

ACKNOWLEDGMENT

The author is thankful to Dr. Anand Chaurasia for his valuable suggestion and support. I also extended our thanks to Dr. Ravindra Pal Singh for his support and motivation. I want to thank Suresh Gyan Vihar University, Jaipur, as well as School of Pharmacy for his support.

REFERENCES

1. Web MD. Skin Problems and Treatments Guide; 2014. p. 132.
2. Patil MB, Jalapure JS, Ashraf A. Preliminary phytochemical investigation and wound healing activity of the leaves of *Argemone maxicana* Linn. Indian Drugs 2001;36:288-93.
3. Bimlesh SK, Prashant P, Manoj S, Kumar SP. A Review of Phytochemistry and Pharmacology of Flavonoids; 2002. p. 162-3.
4. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants used in Ayurveda. Vol. 4. New Delhi: Central Council for Research in Ayurveda and Siddha; 2002. p. 320.
5. Harborne JB. Phytochemical Methods. New York: Chapman & Hall; 1991.
6. Kokate CK. Practical Pharmacognosy. 2nd ed. Pune: Vallabh Prakashan; 2003.
7. Khandelwal KR. Practical Pharmacognosy. Pune: Nirali Prakashan; 2005.
8. Wilson K, Walker J. Principles and Techniques of Biochemistry and Molecular Biology. 7th ed. Cambridge: Cambridge University Press; 2010. p. 476-9.
9. Kumar A, Dutta M, Bhatt TK, Dalal DS. Use of herbal tonic Yakrifit in equine practice. Indian Vet J 1997;74:424-42.
10. Anonymous, The British Pharmacopoeia 2003;33:293-304.
11. Armitage P. Statistical Method in Medical Research. London: Blackwell Scientific Publication; 1971. p. 217.
12. Shirwaikar A, Jahagirdar S, Udupla AL. Wound healing cativity of *Desmodium triquetrum* leaves. Indian J Pharmasci 2003;65:461-4.
13. Bairy KL, Abhinav R, Satyam SM. Evaluation of burn wound healing activity of topical regular insulin in non-diabetic and streptozocin-induced diabetic rats. Int J Pharm Pharm Sci 2014;6:127-30.