"Development, Characterization & Isolation of Phenol Fraction from *Asteracantha longifolia* and Evaluate its Wound Healing Activity"

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

1. *Research scholar, Suresh Gyan Vihar University,Jaipur (Rajasthan),*
2. *Vedica College of B.Pharmacy, RKDF University, Bhopal, M.P (India)*
3. *Suresh Gyan Vihar University, Jaipur (Rajasthan)*

ABSTRACT

Abstract: A wound is break in the skin. Wound are usually caused by cuts or scalps, 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 Development, Characterization & Isolation of Phenolic Fraction from Asteracantha longifolia and Evaluate its Wound Healing Activity in Various wound healing models like Excision, Incision, Dead space wound and Burn wound healing models. Various Evaluation Parameters like wound contraction, Epithelisation time, tensile strength, wet and dry granuloma weight and hydroxiproline 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 like increase the collagen synthesis, fibroblast proliferation, angiogenesis and epithelization, because products which are available in market are either antiseptic or antimicrobial.

Key Words: ***wound, swelling, tenderness, scar***

Introduction

The skin is the most important organ of body which covers total area of about 20 square feet. The skin protects human body from microbes and the elements; helps 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 a important role in photosynthesis. Flavonoids showing various pharmacological activities like anti-inflammatory, antibacterial, antiviral, anti allergic antitumor, treatment of neurodegenerative diseases, wound healing activity of selected plants was performed by using various wound healing models like Incision, Excision, Dead space and burn wound model[3]. *Astercantha Longofolia* commonly known as Kokilaksha belongs to Acanthaceae family. Present study was aimed to Development, Characterization & Isolation of Phenolic Fraction from *Asteracantha longifolia* and evaluates its Wound Healing Activity by using various wound healing models4

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Aerial parts of *Asteracantha long folia,* was collected during the June to August and late September from the surrounding area of Bhopal (MP). The aerial parts and leaves collected were shade dried using tray under controlled temperature at 35 ^{0}C. plants were then drug converted in powder by the help of pulverize and powder was store in polythene bags which free from microbes. The plant material was identified from

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]

Physical specification are to be determined, These are rarely constant for each crude drugs, but may help in evaluation, specifically for moisture content, foreign organic matter,, ash value, extractive value etc.

Qualitative Analysis of Extracts[5.6.7]

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,phenoliccompound Proteins and free amino acids, Flavonoids

Purification by chromatographic techniques

Chromatography is a laboratory techniques 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 verity 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 are 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 Flavonoids[5]

Extraction: 1g powdered plant material was extracted with 10 ml methanol for 5 min., on a water bath at about 60 ^{0}C and filtrate What man filter paper No. 44. It was evaporated to 2 ml, and to this, 1 ml distilled water and 10 ml EtOAc were added and shaken several times. Then, the EtOAc phase was separated and reduced to 1 ml for TLC to separate flavonoids.

Development: 20 µl of each extract was loaded on a percolated Alugram®Sil G/F_{254} with a capillary tube. Then, the plate was kept in a saturated chromatographic chamber containing chloroform and ethyl acetate (8:2) mixture as solvent system.

Detection: The developed chromatographic plates were observed under (UV_{254}nm) chamber. Thus, the colour and hRf values of fluorescent bands obtained were recorded.

Separation of Phenols[5]

Extraction: 2g plant material was dissolve in 10 ml of methanol and allowed to stay overnight on a rotary shaker (180 thaws/min). The filtered extract is evaporated to 1/4th volume and used for separation of phenols

Development: 20 µl of the extract was loaded on the activated chromatographic plates and kept in the saturated chromatographic chamber contain $CHCl_3$ and MeOH (27:0.3) mixture as solvent system.

Detection: The developed chromatograms were observed under visible light after spraying with half diluted FCR and heated at 110 ^{0}C for 10 min. Thus, the phenolic bands obtained colour and hRf values were recorded.

Separation of Alkaloids

Extraction: 100g 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% (v/v) H_2SO_4. The resulted organic phase was separated using separating funnel and basified with NH_4OH (pH 11-12). This is extracted with 1000 ml $CHCl_3$ (3X) and was dried over Na_2SO_4 and evaporated to dryness at 40 ^{0}C in vacuo

Development: 20µl of redissolved alkaloid extracts were loaded with a capillary tube on a precoated Alugram®Sil G/F_{254} plate and was allowed to dry. 20µl brucine (Hi-Media Lab. Ltd., Mumbai) 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 Ultra Violet light (UV_{250} nm) chamber, and recorded the colour and the hRf values of bands. Later, the plate were observed under visible light after spraying with Dragendorff's reagent and heated at 100 ^{0}C for 5 min.

Separation of Sterols: 2 g of powdered fruit samples was extracted with 10 mL methanol in water bath (80^{0}C/15 min). The condensed filtrate was used for chromatography. The sterols were separated using chloroform, glacial acetic acid, methanol and water (64: 34: 12: 8) solvent mixture. The colour of hRf values of these spots were recorded under visible light after spraying the plates with anaisaldehyde Sulphuric acid reagent and heating (100 ^{0}C/6 min)

Separation of active fraction from selected plant solvent extract using Column Chromatography[5]

This technique is based on the differential adsorption of 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 to provide control on the flow rate of mobile phase. The packing material was supported inside the column by in built 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 in order to use this data as the basis in the process identification and structure identification of compounds like Nature Colour hRf values Melting point Solubility Yield. The purified compounds were identify by UV, FT-IR, ^{1}HNMR, 13NMR 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 **(Ahmed *et al.*,1985;Yamaguchi,1970)**

Formulation preparation

Ointments of *Asteracantha longifolia* and its Isolated Phenolic Fraction were prepared by using simple ointment base **BP**. All the doses for the test extract were fixed from the acute toxicity studies (**Kumar Sathish et al.2011)** Topical application of extract was made in the case of excision, incision and burn wound model and dead space wound model receives oral suspension.. For topical administration, 5% w/w of extract ointments was prepared using simple ointment base BP **(Anonymous, 1953)**

Table1: Composition of Simple ointment base for control Group (100 gm)

S.No.	Constituents	Quantity
1.	Polyethylene Glycol 400	40 gm
2.	Polyethylene Glycol 600	60 gm

Formulation of suspension

Suspensions of *Asteracantha longifolia* and its Isolated Phenolic Fraction was prepared by using following formula. Suspension of test drug extracts was prepared by mixing 2 gm of drug with 20 ml of Tragacanth mucilage. Mucilage was prepared by using formula given below. In which purified water added to make it 100gm.

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.

Table2: Composition of Tragacanth mucilage (100 gm)

S.No.	Constituents	Quantity
1.	Glycerin	18 gm
2.	Purified water	75 ml
3.	Tragacanth	2 gm
4.	Benzoic acid	0.2 gm

Wound healing activity

Selection and procurement of animals

After taking permission for animal studies from Institutional Animals Ethics Committee **(TIT/IAEC/831/P'COLOGY/2015/54**,) rats of wistar strain were procured and rats of either sex weighing 150-200 gm were selected, maintained at 24-28^{0}C, housed independently with free access to food and water. The animals were left free for 48 hr. to maintain them in the animal room conditions. Standard pellet diet was given to them. To perform the experiment, the rats were divided into Four groups (n=6) **(**Armitage, 1971) The results were analyzed by one-way ANOVA and a P-value less than 0.01 was considered significant.

Selection of model

Excision, Incision, Dead space, 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 (Shirwaikar et al, 2003)

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 a anesthetic agent. A wound of about 2.5 cm diameter was made which was cerqular 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 (Approx. area 500 mm^2). This was taken as the initial wound area reading. 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, animal of group III Treated as Test II which received ointment of *A.longofolia,* ointment of Phenolic Fraction of *A.longofolia* topically, Group IV contain 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 4th, 8th, 12th and 16th, post wounding days. The wound area of each animal was measured by using tracing paper method. The percentage of wound contraction was calculated from the days of measurements of wound area.

Incision wound model (Shirwaikar et al, 2003)

In this particular wound model Wister rats were selected and they shave by removing hairs at the dorsal thoracic region. Diethyl ether use as a anesthetic agent. Six centimeter long Para vertebral incisions were made through full thickness of skin on either side of vertebral column of the rat. The wounds were closed with interrupted sutures of one centimeter 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, animal of group III Treated as Test II which received ointment of *A.longofolia,* ointment of Phenolic Fraction of *A.longofolia* topically, Group IV contain 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 8^{th} post wounding day. The tensile strength of wounds was measured on 10^{th} day following continuous water flow technique

Dead space wound model (Shirwaikar et al, 2003)

In this particular wound model a grass pith(2.5 cm x 0.3 cm), is selected and sterilized after that this grass pith implant in a dead space wound model by using light ether anesthesia on either side of the dorsal Para vertebral surface of rat. The rats are categorized into four groups (n=6). The animal of group I treated as control and only Suspension base given orally. The animal of group II Treated As TEST I, animal of group III Treated As Test II which received suspension of *A.longofolia,* Suspension of Phenolic Fraction of *A.longofolia* , Group IV contain standard drug. Which received Povidone iodine daily for 16 days, starting from the day of wounding? All the samples were given once daily for 10 days, starting from the day of wounding. On 10^{th} day of wounding granuloma tissue which was formed on grass p pith were excised. The weight of wet and dry granulation tissues was measured along with estimation of biochemical parameter like hydroxyproline estimated.

Dry Granuloma Weight

The granuloma was collected from grass pith at 10^{th} day from dead space wound model. The granuloma was dried at 60˚C for three hour 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 hr at 105º 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 58º 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 (bairy.k.l et al 1997)

Albino rats of wistar strain (150-200gm) body weight were selected and maintained at uniform temperature and diet in well ventilated cages. Medium thick burn wound were inflicted on overnight starved animal under light ether anesthesia using a metal rod(1.5cm in diameter) heated to 80-85^0 c and exposed for 20sec.after 24 hrs dead tissue were excised using sterile surgical blade. Wound contraction was measured after completion the model

Result and Discussion:

Plant products have been part of phyto medicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. . 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 ethno botanical studies, detailed literature survey 03 more effective plants Aerial parts of *Asteracantha longifolia,* was selected because it contain contain various constituents which enhances 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 No 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 No 3** Herbal drugs should be devoid of insects, molds, animal excreta and other contaminants like soil, stone, dust and other extraneous matter like glass pieces, metal parts etc Results are shown in **Table No 3**

S.No.	Parameters	*A.longofolia(%)*
1.	Moisture Content	9.21
2.	Total Ash	7.26
3.	Acid Insoluble Ash	1.23
4	Water soluble ash	3.21

5	Foreign organic matter	0.36

Table No.3. Physicochemical parameters

Extractive value determinations tell us the amount of phytoconstituents in a given amount of medicinal plant material when extracted with a particular solvent. Under a given sent of conditions theses values varies within a narrow limit and hence can be set as an in-house standard for routinely used drugs The results of alcohol soluble and water soluble extractive values are shown in **Table No 4.**

S.No.	**Extractive values**	***A.longofolia(%)***
1.	Alcohol Soluble	12.85
2.	Water Soluble	20.29

Table No. 4: Extractive Values

Plants are known to contain various primary metabolites like carbohydrates, proteins and fats that are consumed as food by animals and humans. They also contain various secondary metabolites like alkaloids, terpenoids, flavonoids, glycosides, tannins etc which shows certain physiological effects. Qualitative phytochemical test were perform on all three selected plants which shown presence of Alkaloids, Tannin, Protein, Amino acids, carbohydrates and flavonoids,saponons,Tannins**.** All the extracts of selected medicinal plant were screened for the presence of different phytoconstituents and results are shown in **Table No 5**

S.No	Compound	*A Longifolia*
1	Steroids	+
2	Glycosides	_
3	Saponin	_
4	Cabohydrates	+
5	Alkaloids	+
6	Flavonoids	+
7	Tannins	+
8	Protein	+
9	Amino acid	+

Table No 05: Qualitative Phytochemical Test

Qualitative separation from *Asteracantha longifolia* by TLC

The TLC of *Asteracantha longifolia* was performed by using chloroform and ethyl acetate (8:2) as a developing solvent and sulphuric acid as a spraying reagent.

The chromatogram of ethanol extract of *Asteracantha longifolia* displayed 4 distinct bands possessing dark blue, yellow, yellow, yellow blue with hRf values 53, 69, 84 and 90, respectively. The chromatogram of ethanol extract of *Asteracantha longifolia* displayed 6 distinct bands possessing light brown, light blue, blue, dark blue, blue and light sky blue with hRf values 06, 07, 10, 20, 22 and 32. The chromatogram of ethanol extract of *Asteracantha longifolia* displayed 3 distinct bands possessing green, blue and light green with hRf values 38, 60 and 88 **(TableNo6)**

Table 6: Qualitative separation of from *Asteracantha longifolia* by TLC

Secondary Metabolites	Number of bands	Rf values	Colour of the bands
Flavonoids	4	53	**Dark blue**
		69	**Yellow**
		84	**Yellow**
		90	**Yellow blue**
Phenols	6	06	**Light brown**
		07	**Light blue**
		10	**Blue**
		20	**Dark blue**
		22	**Blue**
		32	**Light sky blue**
Alkaloids	3	38	**Green**
		60	**Blue**
		88	**Light green**

Separation of Phenols fractions from *Asteracantha longifolia* by the Column chromatography : *Asteracantha longifolia* is a rich source of phenols & flavonoids of pharmacological importance. Thus an attempt was made to isolate some of these phenolic fractions from the Aerial part of *Asteracantha longifolia* by column chromatography and purified with the help of preparative thin layer chromatography. The crude effective extract of *Asteracantha longifolia* about 10 g was fractioned on a Silica gel-H (60-120 Mesh) column at a room temperature and pressure (26 ^{0}C. 1br). After discarding 200 ml dead volume from the column (Hexane), total 44 fractions of 100mleachwerecollecte The fractions 1 to 11 were obtained from the pet ether: chloroform, 12 to 22 was collected from chloroform: ethyl acetate, 23 to 33 was collected from ethyl acetate: methanol. Totally 44 fractions were collected and the concentrated solutions of these fractions had waxy nature. Fractions of 1 to 5, 13, 14, 19,

Sl. No.	Mobile phase	Ratio of Mobile phase	Number of fractions	Colour of the extract	Natureof the Extract
01.	Petether:Chloroform	100:00	1	Light brown mass	Waxy
02.	Petether:Chloroform	90:10	1	Light brown mass	Waxy
03.	Petether:Chloroform	80:20	1	Brown mass	Waxy
04.	Petether:Chloroform	70:30	1	Dark brown mass	Waxy
05.	Petether:Chloroform	60:40	1	Dark brown mass	Waxy

06.	Petether:Chloroform	50:50	1	Light Brown powder	Amorphous
07.	Petether:Chloroform	40:60	1	Brown powder	Amorphous
08.	Petether:Chloroform	30:70	1	Dark brown powder	Amorphous
09.	Petether:Chloroform	20:80	1	Dark brown powder	Amorphous
10.	Petether:Chloroform	10:90	1	Dark brown powder	Amorphous
11.	Petether:Chloroform	00:100	1	Light Brown powder	Amorphous
12.	Chloroform:Ethylacetate	100:00	1	Brown powder	Amorphous
13.	Chloroform:Ethylacetate	90:10	1	Reddish brown mass	Waxy
14.	Chloroform:Ethylacetate	80:20	1	Reddish brown mass	Waxy
15.	Chloroform:Ethylacetate	70:30	1	Reddish brown powder	Solid
16.	Chloroform:Ethylacetate	60:40	1	Reddish brown powder	Solid
17.	Chloroform:Ethylacetate	50:50	1	Dark brown powder	Solid
18.	Chloroform:Ethylacetate	40:60	1	Brown powder	Solid
19.	Chloroform:Ethylacetate	30:70	1	Dark Reddish brown mass	Waxy
20.	Chloroform:Ethylacetate	20:80	1	Dark Reddish brown mass	Waxy
21.	Chloroform:Ethylacetate	10:90	1	Dark Reddish brown powder	Solid
22.	Chloroform:Ethylacetate	00:100	1	Light brown mass	Waxy
23.	Ethylacetate:Methanol	100:00	2	Light brown mass	Waxy
24.	Ethylacetate:Methanol	90:10	2	Dark brown mass	Waxy
25.	Ethylacetate:Methanol	80:20	2	Brown mass	Waxy
26.	Ethylacetate:Methanol	70:30	2	Reddish brown powder	Solid
27.	Ethylacetate:Methanol	60:40	2	Reddish brown powder	Solid
28.	Ethylacetate:Methanol	50:50	2	Brick red powder	Semi-solid
29.	Ethylacetate:Methanol	40:60	2	Dark brownish red powder	Solid
30.	Ethylacetate:Methanol	30:70	2	Dark brown powder	Solid

31.	Ethylacetate:Methanol	20:80	2	Dark brown powder	Solid
32.	Ethylacetate:Methanol	10:90	2	Light blue brown mass	Solid
33.	Ethylacetate:Methanol	00:100	2	Dark blue powder	Solid

Table 7: Isolation of compound fractions through column chromatography

20 and 22 to 25 appeared waxy, 06 to 12 were amorphous and 15 to 18, 20, 26 to 33 Were appeared to be solid (**TableNo7)** The fractions 1 to 11 were obtained from the pet ether: chloroform, 12 to 22 was collected from chloroform: ethyl acetate, 23 to 33 was collected from ethyl acetate :methanol. Totally fractions were collected and the concentrated solutions of these fractions had waxy nature. Fractions of 1 to 5, 13, 14, 19, 20 and 22 to 25 appeared waxy, 06 to 12 were amorphous and 15 to 18, 20, 26 to 33 were appeared to be solid(**TableNo07)** Column chromatography, using petroleum ether-chloroform-ethyl acetate-methanol as mobile phase yielded one major phenolic compound **2-(3-hydroxyphenyl)-3-methoxy-4*H*-furo[2,3-*h*] chromen-8(9*H*)-on**

Ultraviolet spectroscopy of *Asteracantha longifolia*

The λ_{max} for flavonoid was found to be 530 nm respectively, which was conchordant with their respective standard. The UV spectrum and standard curve for the respective constituent was shown in **Fig.1** The organic molecule in solution form when exposed to light in the ultraviolet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption which is the characteristic of the particular constituent or compound **(Harbone, 1973)**. The λ_{max} of isolated phytoconstituents was obtained from UV scan was similar with their respective standard confirms the presence and purity of phytoconstituents.The compounds were dissolved in 5 ml of chloroform (Analytical grade) and read the absorbance in the Perkin-Elmer Lambda 15 UV/VIS spectrophotometer in the ranger of 200-800 nm wavelengths against chloroform blank. The plotted graph i.e abscissa verses ordinate (λ max) is used in the detection of chromophore of the co

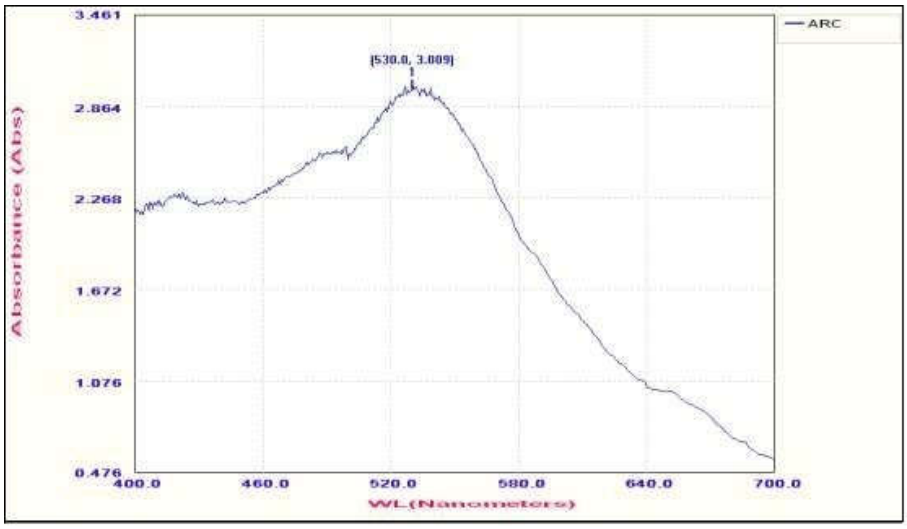


Figure 1: U.V Spectrum of *Asteracantha longifolia*

Fourier Transform Infra Red Spectroscopy *Asteracantha longifolia*

The Fourier transform infrared (FTIR) spectrum of any compound or drug gives information about the functional groups present in that particular compound. Fundamentally, each type of bond in a molecule will absorb a characteristic frequency of IR light as it vibrates, and these frequencies can often be used to determine which types of bonds the molecule actually contains. These vibrations fall into several different categories as shown below for a generic three atom system. In IR spectroscopy, wavelengths is measured in microns (µm) and frequency is measured in wave numbers (cm^{-1})In general, the symmetric bond stretching frequencies are more clearly shown in the IR spectrum and found in the 4000-1500 cm^{-1} region. The other types of vibrations, ant symmetric stretches and scissorings, are generally seen in a complex region below 1500 cm^{-1} called the "fingerprint region" **(Sherman, 2004; Kalsi, 2005)**.The Fourier transform infrared spectra of pure compound were obtained using KBr discs on Perkin-Elmer RX1 spectrophotometer in the wave numbers (cm^{-1}) in the range of 4000-4500 cm^{-1} was recorded as the inverted peaks. 3430cm^{-1}, OH Stretching. 3002cm^{-1}, C-H Stretching. 1660cm^{-1}, Carbonyl stretching **Fig No2**

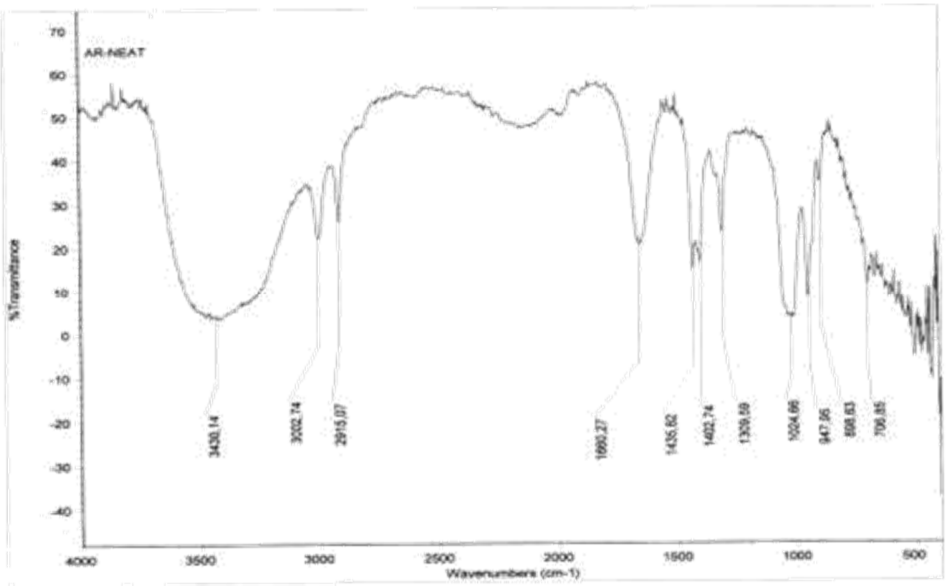
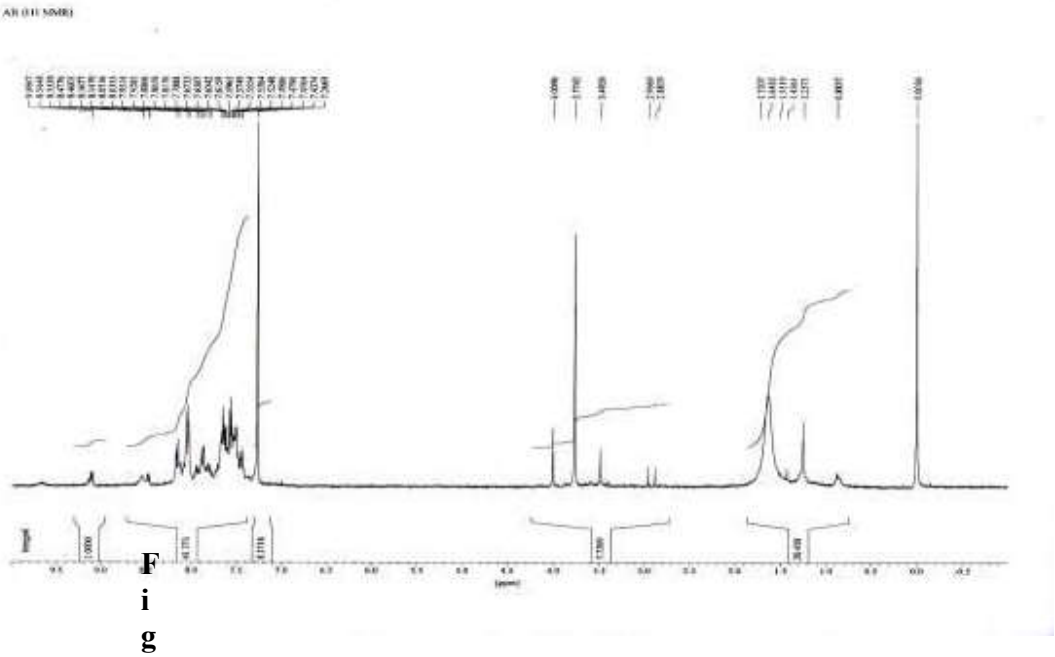


Figure 2: Infra-Red Spectrum of *Asteracantha longifolia*

^{1}H Nuclear magnetic resonance spectroscopy (NMR)

The pure isolated compound spectra were recorded in JEO1 Model GS x 400 spectrophotometer CDCL3 (denatured chloroform, DMSO) was the solvent. ^{1}H NMR was recorded in the Bruker AM X 400 NMR spectrophotometer using TMS (Tetra methyl saline) as an internal standard at 400.137 (1H) and 270 c or 300 K. The chemical shift were recorded in σ (PPM) based either on σ TMS=0 and the coupling contestants or J in hertz. **Fig No 3,4,5**



Fig

Figure 3: ^{1}HNMR of *Asteracantha longifolia*

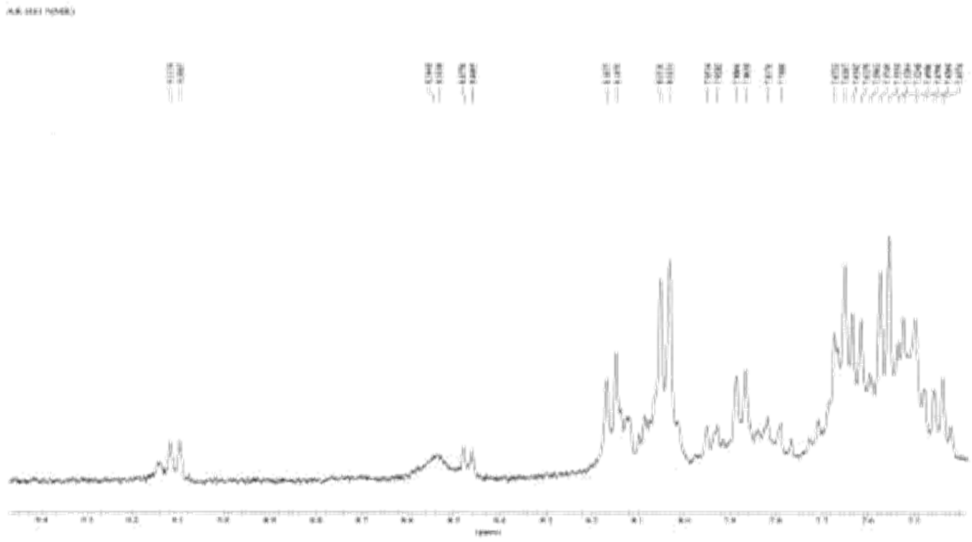


Figure 4: ^{1}HNMR of *Asteracantha longifolia*

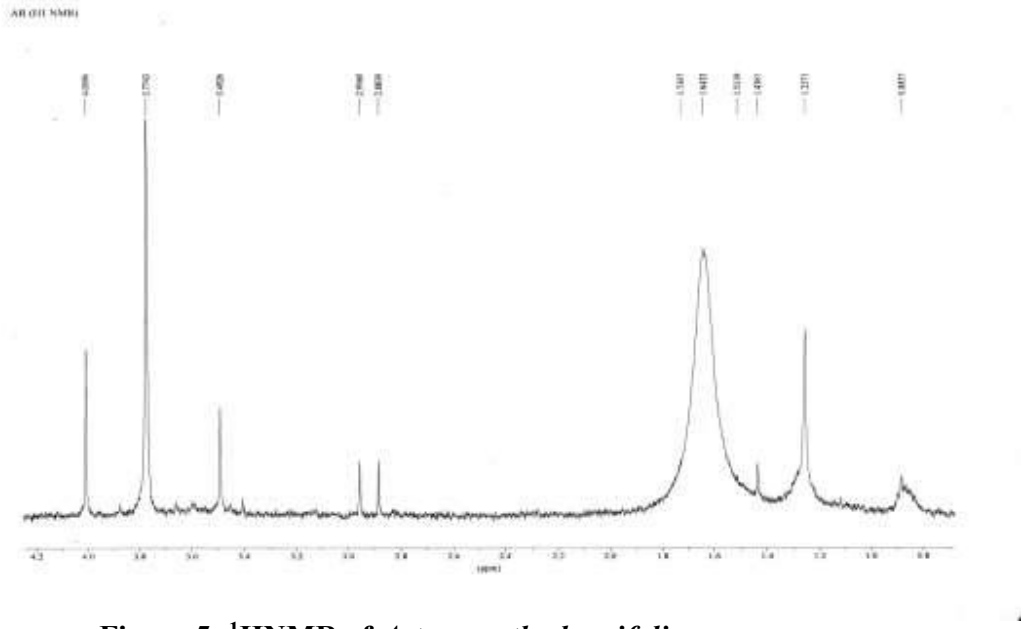
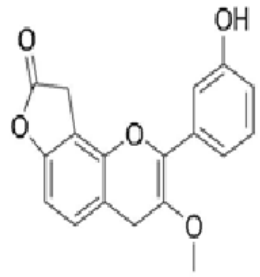


Figure 5: ^{1}HNMR of *Asteracantha longifolia*

characterization of isolated compound (AL): *Asteracantha longifolia* extract was obtained with 12.2% w/w yield and Based on the physico-chemical properties, the active compound (Al) is identified as **2-(3- hydroxyphenyl)-3-methoxy-4*H*-furo [2,3-*h*]chromen-8(9*H*)-one,** Which has properties like **Nature:** Solid, **Colour:** Dark blue powder, **hRf value:** 22, **Melting point:** 156-158^{0}C, **Solubility:** Water, dilute acids, alkalies. DMSO and DMF.**UV:** 530nm, **IR:** 3430cm^{-1}, OH Stretching. 3002cm^{-1}, C-H Stretching. 1660cm^{-1}, Carbonyl stretching.,**^{1}HNMR**: δ 9.06, (s, OH), δ 8.54-7.43 (m, 6Ar-H), δ 4.0(s, 2H), δ 3.77 (s, 3H), δ 3.49
(s, 2H



2-(3-hydroxyphenyl)-3-methoxy-4*H*-furo[2,3-*h*]chromen-8(9*H*)-one

Figure 6: Separation of Phenols from *A.L* by Column chromatography

Wound healing activity:

Selection and procurement of animals

After taking permission for animal studies from Institutional Animal Ethical Committee **(TIT/IAEC/831/P'COLOGY/2015/54** Wistar albino rats were procured and male rats weighing 150-200 gm were selected, maintained at 24-28^{0}C, housed individually with free access to food and water. The animals were left for 48 hr. 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, standard drug and for Dead space wound model four groups were prepared which and divided in control, Test I, Test II, standard drug.

Wound Contraction and Epithelization time in Excision wound model: The wound contraction was calculated as 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 for scar to fall off leaving no raw wound behind. Effect of control, Test I, Test II, standard drug (Povidone Iodine) was observed on percentage wound contraction in Excision wound model on Initial, 4th, 8th, 12th, 16th day interval which is shown in **Table No 8**.It has been seen that significant wound healing took place in case of animals treated with ointment of Phenolic fraction of *Astercantha Longifolia* which is 17 days and ointment of Extract of *Astercantha Longifolia* took 21 days for complete wound healing. The least rate of wound healing was seen in control group which received no treatment and fastest rate of wound healing was seen in standard drug group where animals received standard drug which is Povidone iodine .Epithelization period (days) in excision wound healing model also shown in **Graph No 1**.

	Area of wound closure (sq mm ± S.E.M)					
Groups	**Initial**	**4th day**	**8th day**	**12th day**	**16th day**	**Epithelization period (Days)**
I (CONTROL)	10.82±0.68	18.82±0.68	38.12±1.80	48.21±1.80	68.69±2.60	24
II (A.L extract)	11.24±1,23*	24.22±1.42*	48.94±1.24*	66.92±0.13*	76.12±1.93*	21
III (Isolate of A.l)	14.16±1.05*	30.29±0.25*	50.16±1.25*	80.45±1.95 *	95.12±0.05*	17
IV (Standard drug)	15.26±1.25*	42.24±1.07*	65.34±1.70*	90.12±1.08*	100±0.75*	16

Table 8: Mean Percentage wound contraction in Excision wound model

Initial wound area approx. 500 sq mm, ≈ n = 6 animals in each groups.

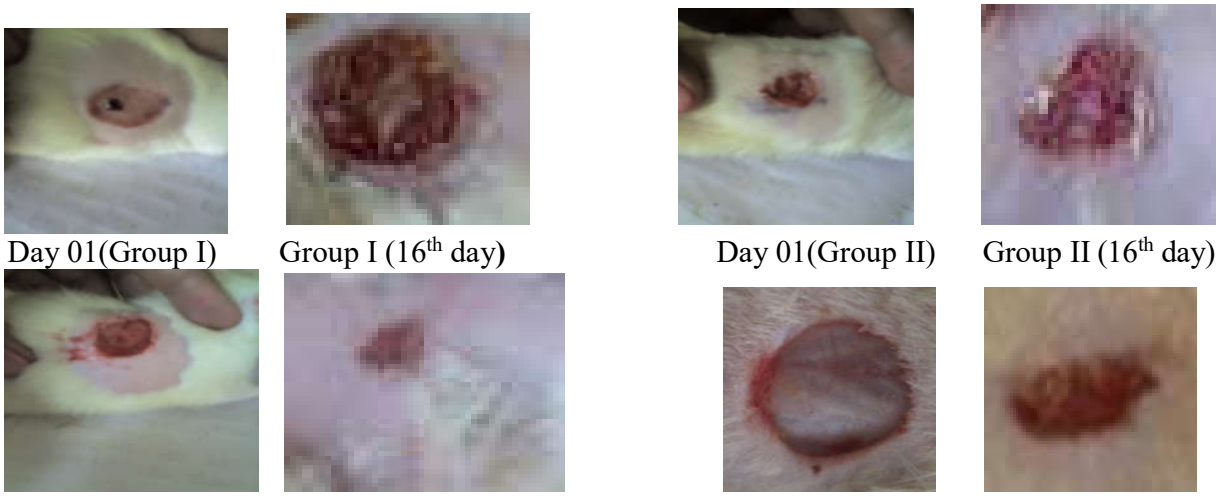
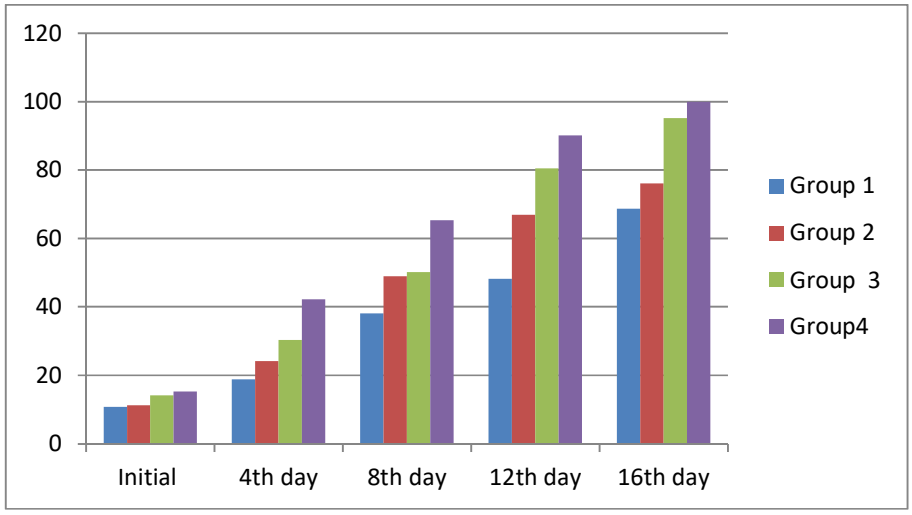


Fig 7: Wound contraction in Excision wound model (Ist and 16th day)



Graph1: Epithelization period (days) in Excision wound healing model

Measurement Tensile strength in Incision wound model

The tensile strength was calculated in incision wound model. On 10th day the rats were again anesthetized and each rat is placed on a stack of paper towel on the middle of the board Effect of control, Test I, Test II, standard drug (Povidone Iodine) was observed on Tensile strength in Incision wound model which is shown in **Table No9** which indicate that animals treated with Ointment of Phenolic fraction of *Astercantha Longifolia* shows significant higher Tensile strength As compare to extract of *Astercantha Longifolia*. The least tensile strength seen in control where animal not received any treatment and highest Tensile Strength seen in standard drug group where animals received standard drug**.**

Groups	Tensile strength (in Grams)
Control	225.16±3.51
II (A.L extract)	340.39±2.40
III (Isolate of A.L)	410..39±1.20
Standard	490.50±2.71

Table9: Tensile Strength in incision wound model

≠ Result expressed as Mean Area ± S.E.M. (Standard Error Mean), * P≤ 0.01 indicates significant when compared with control

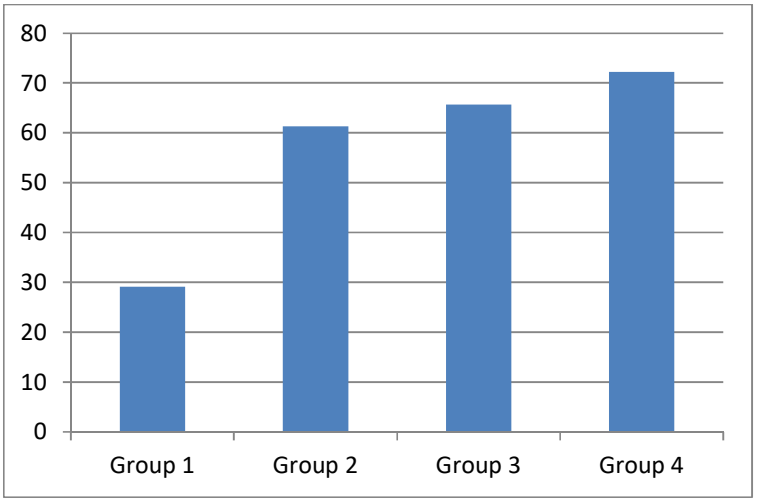
Wet Granuloma, Dry Granuloma Weight and Hydroxiproiline Measurement for Dead space wound model:

Effect of control, *Astercantha Longifoli* suspension, Phenolic Fraction of *Astercantha Longifoli* and standard drug (Povidone Iodine) was observed on Dry, Wet granuloma weight and Hydroxyproline estimation in Dead space wound model which is shown in **Table No 10** which indicate that animals received suspension of Phenolic Fraction of *Astercantha Longifol* shows significant higher Wet granuloma weight (mg/100gm,Dry granuloma weight (mg/100gm), Hydroxiproline (mg/gm of tissue) Estimation as compare to suspension of extract of *Astercantha Longifoli* , control group where animals not received any treatment shown least Wet, Dry granuloma weight and Hydroxiproline estimation and animals received standard drug (Povidone iodine) shown highest Wet, Dry granuloma weight and Hydroxiproline estimation. **Graph No 2, 3, 4** indicate effect of extract on wet granuloma weight, dry granuloma weight and on Hydroxiproline estimation

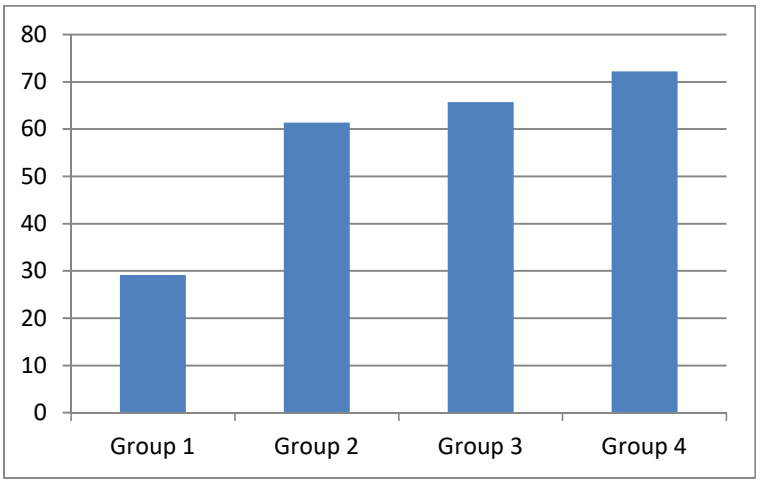
Group(n)	Wet granuloma weight(mg/100gm	Dry granuloma weight(mg/100gm)	Hydroxiproline(mg/gm of tissue)
Control	29.12.±1.20	20.71±3.20	34.70±4.92
II (A.L extract)	61.36±0.91	46.22±0.82	40.20±1..62
III (Isolate ofA.L)	70.23±1.91	56.22±1.82	59.22±0..62
Standard	72.19±1.66	57.62±2.12	69.35±3.22

Table 10: Dry, Wet granuloma weight and Hydroxyproline estimation in Dead space wound healing model

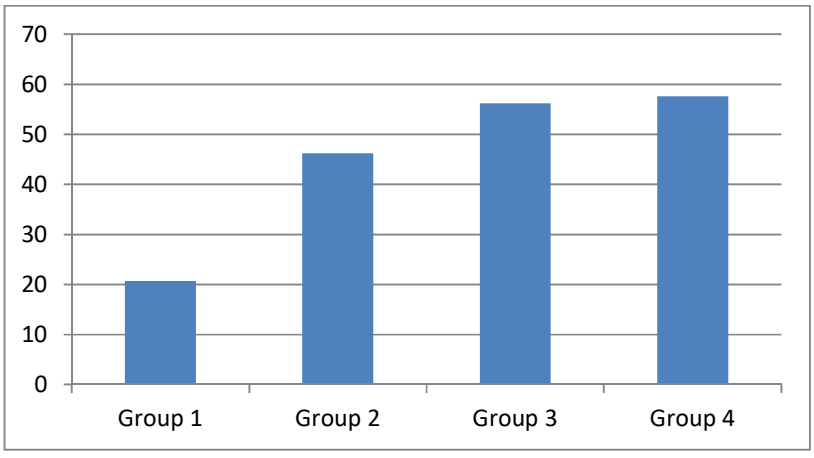
≠ Result expressed as Mean Area ± S.E.M. (Standard Error Mean), * P≤ 0.01 indicates significant when compared with control



Graph2: Effect of extract on wet granuloma weigh



Graph3: Effect of extract on dry granuloma weight



Graph4: Effect of extract on Hydroxiproline estimation

Wound Contraction and Epithelization time in Burn wound model

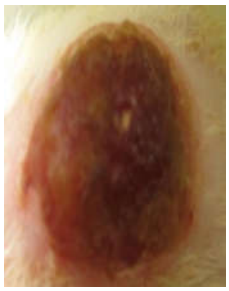
Effect of control, Test I, Test II, Test III, polyherbal formulation, standard drug (Povidone Iodine) was observed on percentage wound contraction in Burn wound model on Initial,4th,8th,12th,16th day interval which is shown in **Table No11** which indicate that faster wound contraction took place in case of animals treated with Polyherbal Formulation which is 18 days and Test drug I took 19 days, Test drug II took 21 days, Test drug III took 22 days for complete wound healing. The least rate of wound healing was seen in control group which received no treatment and took 24 days for complete healing and fastest rate of wound healing was seen in standard drug group where animals received standard drug which is silver sulphadiazain which took 17 day for complete healing**.** Percentage wound contraction in burn wound model shows in **Graph No 5, 6**

	Area of wound closure (sq mm ± S.E.M)					
Groups	**Initial**	**4th day**	**8th day**	**12th day**	**16th day**	**Epithelization period (Days)**
I (CONTROL)	5.92±0.72	20.19±0.92	40.22±1.80	60.11±1.21	70.19±1.20	24
II (A.L extract)	10.14±1.22	30.11±1.22	42.94±1.11	60.12±0.66	69.12±1.29	21
III (Isolate ofA.L)	10.14±1.22	32.11±0.22	53.94±1.21	70.22±1.66	92.12±1.29	19
VI (silve sulphadiazain)	12.13±2.12	40.24±1.24	66.12±1.29	91.92±0.92	99.19±0.71	17

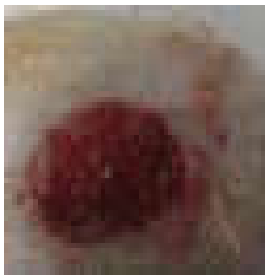
Table 11: Percentage wound contraction in Burn wound model

Initial wound area approx. 500 sq mm, ≈ n = 6 animals in each groups.

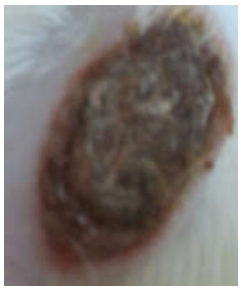
≠ Result expressed as Mean Area ± S.E.M. (Standard Error Mean), * P≤ 0.01 indicates significant when compared with control.



Day01 (Group I)

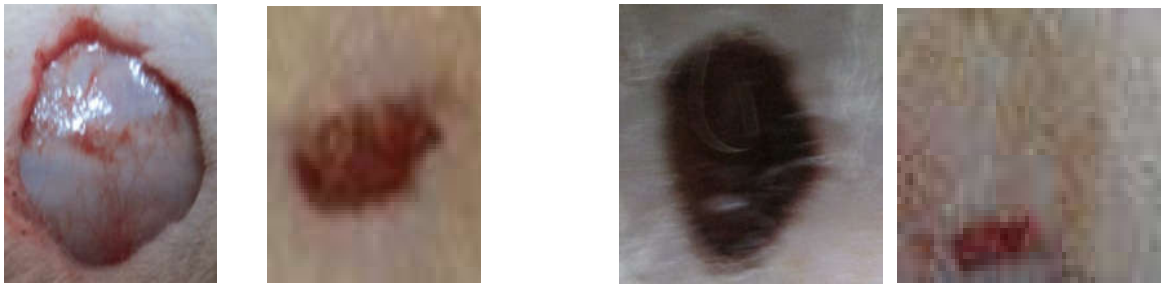


Day16(Group I)



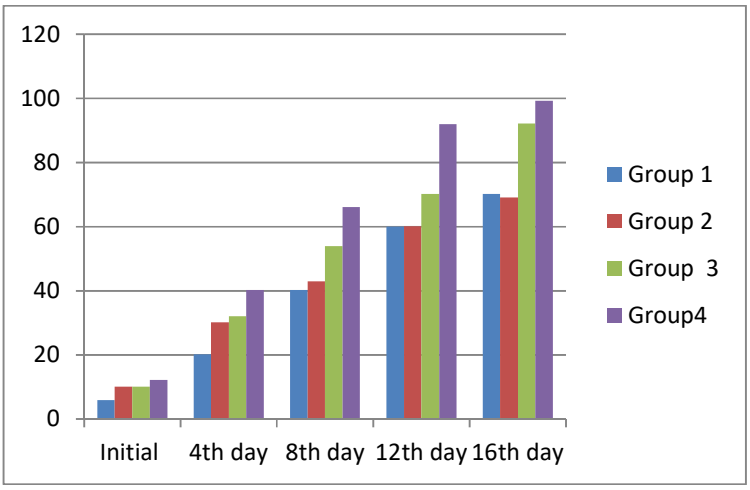
Day01(Group II)

Day01(Group II)

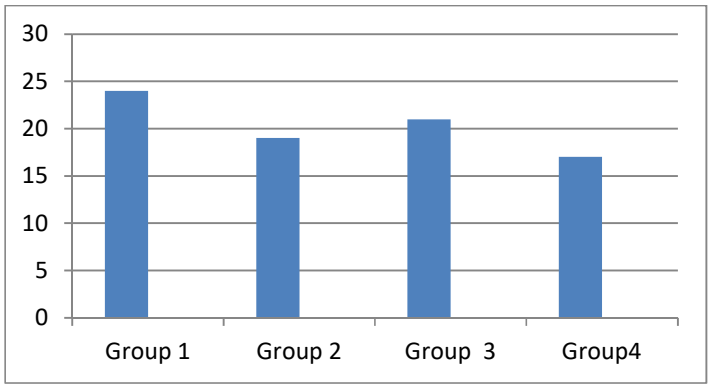


Day01 (Group III) Day16(Group III) Day01 (Group IV) Day16 (Group IV)

Fig 8: Wound contraction in Burn wound model (Ist and 16th day)



Graph 5: percentage wound contraction in burn wound model



Graph 6: Epithelisation period (days) in burn wound model

Conclusion

The skin is a very important organ of human body which covers approx 20 square feet of human body. The skin provides defensive mechanism for body against various microbes, infection and elements. It regulates body temperature. Flavonoids show various biological activities. These includes: anti-inflammatory, antibacterial, antiviral, anti allergic, anti tumor, treatment of neurodegenerative diseases, vasodilatory action. Flavonoids are inhibiting lipid-peroxidation,

platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation these are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, CAMP phosphor diesterase, lipase, α-glucosidase, kinase. **(Kumar Bimlesh et al 2011)** Flavonoids Enhances wound healing activity which proves by various researches. Based on the present investigation, it was well understood that Phenolic fraction of *Astercantha Longifolia* shows significant wound healing activity in all selected wound healing models as compare to standard drug. It was also found that *Astercantha Longifolia* extract and its phenolic fraction shows increasing wound contraction and epithelisation time, increasing tensile strength and increasing wet and dry granuloma weight.

Acknowledgment:

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

References:

1. *Web MD, Skin problems & treatments guide, 2014;132*
2. *Patil M B, Jalapure J S, Ashraf A, preliminary phytochemical investigation and wound healing activity of the leaves of Argemone maxicana linn, Indian drugs,2001;36(6);288-293*
3. *Bimlesh, Sandhar Kaur, Prasher Prashant, Salhan Manoj ,Sharma Pardeep Kumar, A Review of Phyto chemistry and Pharmacology of Flavonoids, 2002;162-3*
4. *P.C Sharma, M B Yelne and T J Dennis, Database on Medicinal Plants Used in Ayurveda, Vol 4, 2002, 320*
5. *Harborne JB. Phytochemical Methods. New York: Chapman & Hall; 1991.*
6. *Kokate C. K. Practical Pharmacognosy.II ed. Pune,Vallabh prakashan, 2003*
7. *Khandelwal K.R, Practical Pharmacognosy, Nirali prakashan (2005), Pune*
8. *Kumar A, Dutta M, Bhatt T.K, Dalal D.S, Use of herbal tonic Yakrifit in equine practice, Indian Veterinary Journal 1997,74, 424-42*
9. *Anonymous, The British Pharmacopoeia 2003, 33(2),293-304.*
10. *Armitage P,Statistical method in medical research,Blackwell scientific publication London,1971,217*
11. *Shirwaikar A, Jahagirdar S, Udupla A L,wound healing cativity of Desmodium Triquetrum leaves,Indian j Pharmasci,2003,65(5),461-464*
12. *Bairy K. L Abhinav R, Shakta mani satyam, evaluation of burn wound healing activity of topical regular insulin in non-diabetic and streptozocin-induced diabetic rats, International Journal of Pharmacy and Pharmaceutical Sciences,2014, Vol 6, Issue 8,127-130*