RESEARCH PAPER

"Phytochemical Profiling and Comparative Evaluation of Wound Healing Activity of *Selaginella bryopteris*, *Jasminum mesnyi*, *Amaranthus spinosus* and their Polyherbal Formulation in Experimental Wound Models

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Abstract: A wound refers to a disruption in the normal continuity of the skin or underlying tissues, which may result from cuts, lacerations, abrasions, or other forms of physical trauma. Wounds may present with symptoms such as swelling, stiffness, tenderness, changes in skin coloration, tightness, itching, and scar formation. Tissue injuries can be broadly categorized into two major types. Wound healing is a complex and coordinated biological process that progresses through distinct phases, including inflammation, tissue proliferation, and remodelling.

The present study is designed to investigate the phytochemical constituents and assess the wound healing potential of *Selaginella bryopteris*, *Jasminum mesnyi*, *Amaranthus spinosus*, and their polyherbal formulation using different experimental wound models. The healing efficacy of the selected plant extracts and polyherbal formulation will be evaluated through parameters such as percentage wound contraction, epithelization period, and tensile strength of the regenerated tissue.

The present investigation aims to perform phytochemical screening and evaluate the wound healing activity of *Selaginella bryopteris*, *Jasminum mesnyi*, *Amaranthus spinosus*, and their polyherbal formulation using various wound models. The wound healing potential will be assessed using various evaluation parameters, including percentage wound contraction, period of epithelization, and tensile strength of the healed tissue.

Key words: wound, abrasions, inflammation, phytochemical screening epithelization,

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Introduction: The skin is the largest organ of the human body, covering an area of approximately 20 square feet. It serves several essential functions, including protecting the body from microbial invasion, maintaining body temperature, and facilitating sensory perception such as touch, heat, and cold. The skin contains various specialized cells, including melanocytes, which are responsible for producing melanin, the pigment that determines skin coloration.

Any disruption, cut, abrasion, or damage to the epidermal layer of the skin may result in the formation of a wound. Common manifestations of wounds include inflammation, stiffness, tenderness, changes in skin coloration, scab formation, itching, and scar development. **Partial-thickness injuries** primarily involve the superficial layers of the skin, including the epidermis and superficial dermis, without significant damage to the underlying blood vessels. Such injuries generally heal rapidly through the process of tissue regeneration.

In contrast, **full-thickness injuries** extend into the deeper dermal layers and may cause damage to the dermal blood vessels. Consequently, the healing process is generally slower and involves the formation and remodeling of new tissue. Burn injuries are among the most severe and painful forms of tissue injury and may affect both the skin and deeper tissues. Burns can result from exposure to hot liquids or solids,

flames, corrosive chemicals such as sulfuric acid, electricity, or radiation. Treatment approaches vary depending on the severity and cause of the burn.

Wound healing is a complex and highly coordinated biological process involving a continuous sequence of events, including hemostasis, inflammation, granulation tissue formation, tissue repair, and maturation. Various cellular components, including epithelial cells, endothelial cells, platelets, and fibroblasts, contribute to tissue repair and restoration of the injured area. A proper understanding of the physiology of wound healing is essential for developing effective therapeutic approaches and improving the overall healing process[1,2].

The primary objective of the present study is to conduct phytochemical screening and evaluate the wound healing activity of *Selaginella bryopteris*, *Jasminum mesnyi*, *Amaranthus spinosus*, and their polyherbal formulation using experimental excision and incision wound models

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Material & Methods:

Plant Material: Whole plant of *Selaginella Bryopteris,* roots of *Jasminum Mesnyi* and leaves *Amaranthus Spinosus* (were collected during the June to August from the surrounding area of Bhopal (MP). The collected parts were shade dried using tray under controlled temperature at 35^0C. Plants were then converted in powder by the help of pulverizer and powder was store in polythene bags which free from microbes.

Extraction: Extraction were done by using successive solvent extraction method i.e. soxhlation. The crude drug was first converted in to powder and then by using pet ether they were defatted. Successive extraction was performed by using different solvent as increasing their polarity. Methyl alcohol was selected for final extraction. The extracts obtained were evaporated until dryness at 40 ^{0}C and stored the dried extract at 4 ^{0}C in the refrigerator until further use

Qualitative Analysis of Extracts [3,4,5] 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 Glycosides, Sugar, Phytosterols, Tannins and phenolic compound, 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. It contains two phases one is known as mobile phase and selected extract dissolve in mobile phase which run on the stationary phase. The different compounds of the extract run at different speeds, causing them to separate and the separation based on differential partitioning between these two phases[4] Chromatography may be preparative or analytical. Preparative chromatography is 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 Varity 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 Flavonoids [6]

Extraction: Alcohol was used for the extraction of material and filter-by-filter paper. It was evaporated to 2 ml and add water and EtOAc 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 precoated plate with a capillary tube the plate was placed in a glass chamber containing chloroform and ethyl acetate (8:2) mixture as solvent system.

Detection: The developed chromatographic plates were observed under (UV_{254}nm) chamber.

Separation of Phenols[7]

Extraction: 2g dry powder drug 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 [6, 8.]

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% H2SO4. The resulted organic phase was separated using separating funnel and basified with NH_4OH. This is extracted with 1000 ml $CHCl_3$ and was dried over Na_2SO_4 and evaporated to dryness at 40 ^{0}C in vacuum

Development: 20µl of redissolved alkaloid extracts were loaded with a capillary tube on a percolated plate and was allowed to dry. 20µl 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 Ultra Violet light (UV_{250} nm) chamber and recorded the color and the hRf values of bands.

Separation of Sterols: 2 g of powdered samples was extracted with 10 mL methanol in water bath. The filtrate was used for chromatography[9]

Formulation of ointment[9]

Four Ointments were prepared for all three selected drugs and for poly herbal formulation by using simple ointment base **BP**. All the doses for the test extract were fixed from the acute toxicity studies Topical application of extract was used in excision, incision and burn wound model and dead space wound model receives oral suspension. 5% w/w of extract ointments was used during the study.[10]

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

Table 1: Composition of Simple ointment base for control Group (100 gm)

Formulation of suspension

Four suspensions were prepared for all three-plant extract and poly herbal formulation 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

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

Table 2: Composition of Tragacanth mucilage (100 gm)

Wound healing activity

Selection of animals

After taking approval from Institutional Animals Ethics Committee **(BMRL/IAEC/2026/05-14**,) rats of Wistar strain were selected. They maintained at 26-30^{0}C, housed independently. The animals were left free for 48 hr to maintain them in the normal room conditions. Standard pellet diet was given to them. To perform the study Nine groups were prepared (n=6) [6]

Selection of model

Various models were used by the help of rats for performing the selected activity. the parameters for the Identification of wound healing activity are wound contraction, epithelization, tensile strength, and granuloma weight and hydroxyproline estimation.

Dead space wound model [10,11,12]

In this particular wound model, grass pith (2.5 cm x 0.3 cm) is selected and sterilized after that this grass pith implant in a dead space wound by using light ether anesthesia on dorsal par vertebral area of rat. The rats are categorized into six (n=6). The animal of group I treated as control and received one ml of 2 % tragacanth solution, orally. The animal of group II, III and IV, treated as Test I, Test II, Test III and suspension of *Selaginella Bryopteris Jasminum Mesnyi ,Amaranthus Spinosus* given orally, Group V contain polyherbal formulation and Group VI contain standard drug. All the samples were given once daily for 10 days. On 10th day of wounding granuloma tissue which was formed on grass pith were excised.

Dry Granuloma weight

The granuloma was collected from grass pith at 10th day from dead space wound model. The granuloma was dried at 60˚C for three hour and weighted.

Hydroxyproline estimation in Dead space model.

Procedures:

The tissue, which accumulates granuloma, was collected for the quantification of hydroxyproline. Calculated quantities of tissue sample were immersed in 2 mL of hydrochloric acid, 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 normal 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 [13]

Albino rats (150-200gm) 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 heated to 80-85^0 c and exposed for 20sec.after 24 hrs dead tissue were excised using sterile surgical blade.

Wound contraction; The wound area reduce as the days goes and reduction in wound area known as contraction. Epithelization time means days required for complete recovery without any scar. Sizes of the wounds were noted on a transparent paper every alternate day.

$$\%\text{Wound contraction} = \frac{\text{Initial wound size-specific day wound size}}{\text{Initial wound size}} \times 100$$

Epithelialization

It was actual days needed for the complete recovery of burn wound without scar. Wound contraction mean percentage reduction in wound size at every 4 days interval.

Result & Discussion

Phytochemical Evaluation Plants are known to contain various primary metabolites like sugar, fats which are used by animals and humans. They also contain many secondary metabolites which shows certain physiological effects. Qualitative phytochemical test was performed on all three selected plants which shown presence of various metabolites. All the extracts of selected medicinal plant were undergone for chemical test and results are shown

in **Table 09**. Performed phytochemical tests were proving that all three selected plants contain flavonoids, Alkaloids and Steroids which enhance wound healing activity

Qualitative separation from *Amaranthus Spinosus* by TLC

The Thin layer chromatography of *Amaranthus Spinosus* was done by using silica gel G as an stationary phase , Methanol: chloroform (1:5) and Toluene: Acetone: Acetic Acid (9:1:0.5) as a developing solvent and sulphuric acid as a spraying reagent. Compounds are detected in iodine chamber and Rf values were determined, four spots

S.No	TEST	*Selaginella Bryopteris*	*Jasminum Mesnyi*	*Amaranthus Spinosus*
1	**Steroids**	**-**	+	+
A	Salkowski Test	-	+	+
2	**Glycosides**	+	+	+
A	Bontrager	+	+	+
B	Kellar killiani	+	+	+
C	Legals	+	+	+
3	**Saponin**	+	+	+
A	Foam test	+	+	+
B	Hemolysis Test	+	+	+
4	**Carbohydrates**	+	+	+
A	Molisch Test	+	+	+
B	Barford's Test	+	+	+
C	Fehling Test	+	+	+
5	**Alkaloids**	+	**-**	+
A	Mayers	+	-	+
B	Wagners	+	-	+
C	Dragondroff	+	-	+
D	Haggers	+	-	+
6	**Flavonoids**	+	+	+
A	Shinoba	+	+	+
B	L. acetate	+	+	+
c	Pew's Test	+	+	+
d	NAOH Test	+	+	+
7	**Tannin**	+	+	+
a	Ferric choloride	+	+	+
b	Gelatin	+	+	+
8	**Protein**	+	**-**	+
a	Precipitation test	+	-	+
b	Xanthoproteic	+	-	+
9	**Amino acid**	+	**-**	+
a	Ninhydrine	+	**-**	+

Table 09: Qualitative Phytochemical Test

for Amaranthus spinosus L., which represents the presence of several important compounds such as alkaloids, flavonoids, terpenoids **(Table 13)**

Extract	Mobile Phase	Rf value
	Methanol: Chloroform (1:5)	0.66, 0.58, 0.30

Methanol extract of *Amaranthus spinosus* L	Toluene: Acetone Acetic acid (9:1:0.5)	0.59

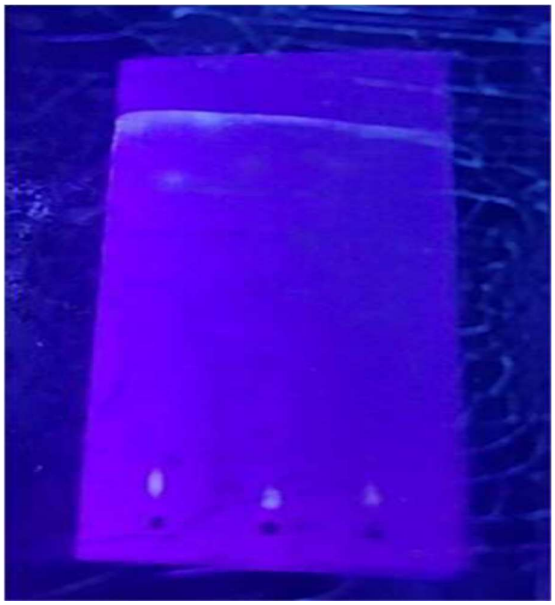
Table 13: Qualitative separation from *Amaranthus Spinosus*

Qualitative separation from *Selaginella Bryopteris* by TLC

The thin layer chromatography confirmed the presence of different bio active components the result and observation were summarized in (table 5.21) and all the solvent different Rf value the chromatograms were visualized under UV light. The Rf value obtained are as follow

Extract	Mobile Phase	Rf value
Chloroform: methanol (75: 25)	7	0.234, 0.49, 0.62, 0.71, 0.79, 0.82, 0.94
Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27)	6	0.661, 0.78, 0.88, 0.92, 0.94, 0.97

Table 14: Thin layer chromatography of *Seleginella Bryopteris*



Qualitative separation from *Jasminum Mesnyi* by TLC

The Thin layer chromatography of *Jasminum Mesnyi* was performed by using chloroform and ethyl acetate (8:2) as a developing solvent and sulfuric acid as a spraying reagent.

The following secondary metabolites from therapeutically important *Jasminum Mesnyi* were separated through thin layer chromatography using chloroform and ethyl acetate (8:2). The hRf values and characteristic colours of the bands were recorded

The chromatogram of methanol: water (7:3) extract of *Jasminum Mesnyi* displayed 4 distinct bands of Flavonoids possessing dark blue, yellow, yellow, yellow blue with hRf values 51, 56, 66 and 80(Table 15)

The chromatogram of methanol: water (7:3) extract of *Jasminum Mesnyi* displayed 3 distinct bands of Phenols possessing Light blue, blue, light sky blue with hRf values 09, 14 and 20.

The chromatogram of methanol: water (7:3) extract *Jasminum Mesnyi* displayed 3 distinct bands of alkaloids possessing green, green and light green with hRf values 36, 59 and 68

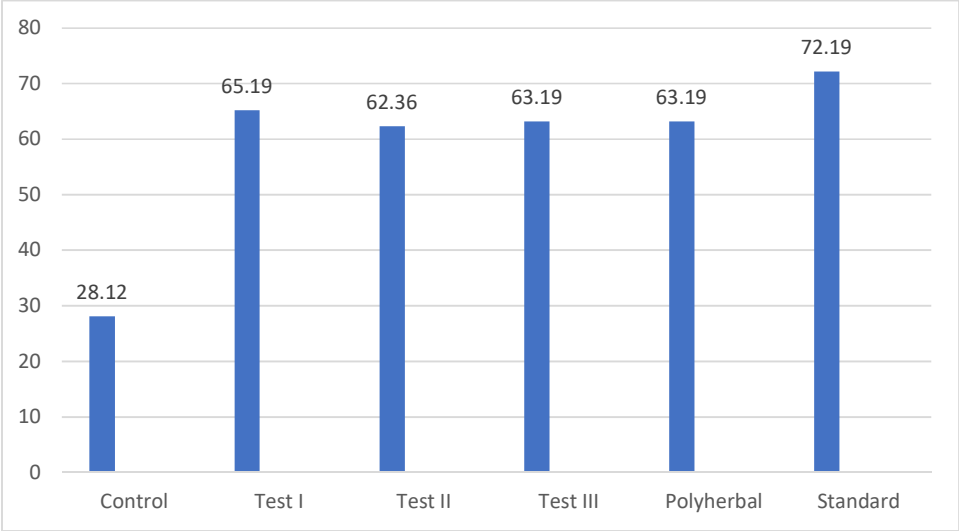
The chromatogram of methanol: water (7:3) extract of *Jasminum Mesnyi* displayed 2 distinct bands of Steroids possessing Light green, greenish black with hRf values 08, and 66

Wet Granuloma, Dry Granuloma Weight and Hydroxyproline Measurement for Dead space wound model: Effect of control, Test I, Test II, Test III, polyherbal formulation, standard was observed on Dry, Wet granuloma weight and proline estimation which shown in **Table 3** which indicate that animals received suspension of Polyherbal formulation shows highest Wet, Dry granuloma weight and proline estimation. Test drug I, Test drug III, Test Drug II, 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 animal shows highest Wet, Dry granuloma weight and proline estimation. **Graph 1, 2, 3** indicate effect of extract on wet, dry granuloma weight and on proline estimation

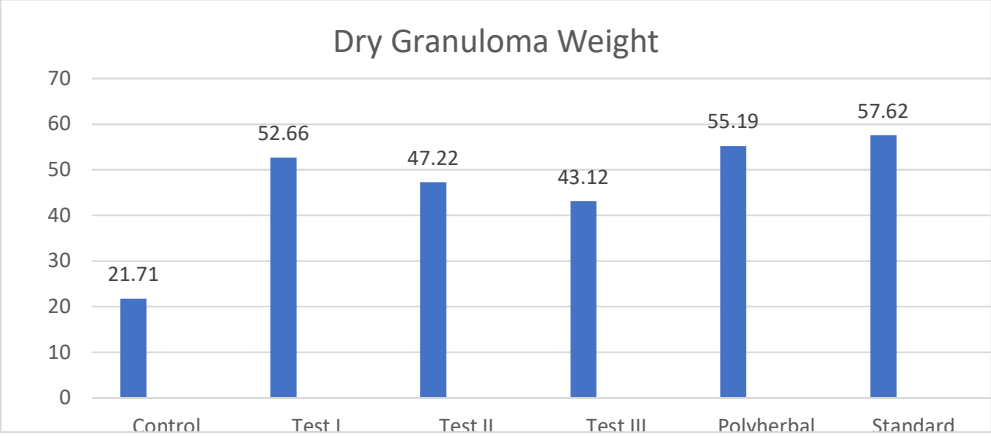
Group(n)	Wet granuloma	Dry granuloma	Hydroxyproline (mg/gm of tissue)
Control	28.12.±1.20	21.71±3.20	35.70±4.92
Test I	65.19±2.12	52.66±1.23	56.12±3.23
Test II	62.36±0.91	47.22±0.82	41.20±1..62
Test III	63.19±0.82	43.12±1.93	39.21±2.12
Polyherbal	66.66±2.22	55.19±1.22	46.20±1.43
Standard	72.19±1.66	57.62±2.12	69.35±3.22

≠ Result expressed as Mean Area ± S.E.M. * P≤ 0.01 shows importance when compared with control

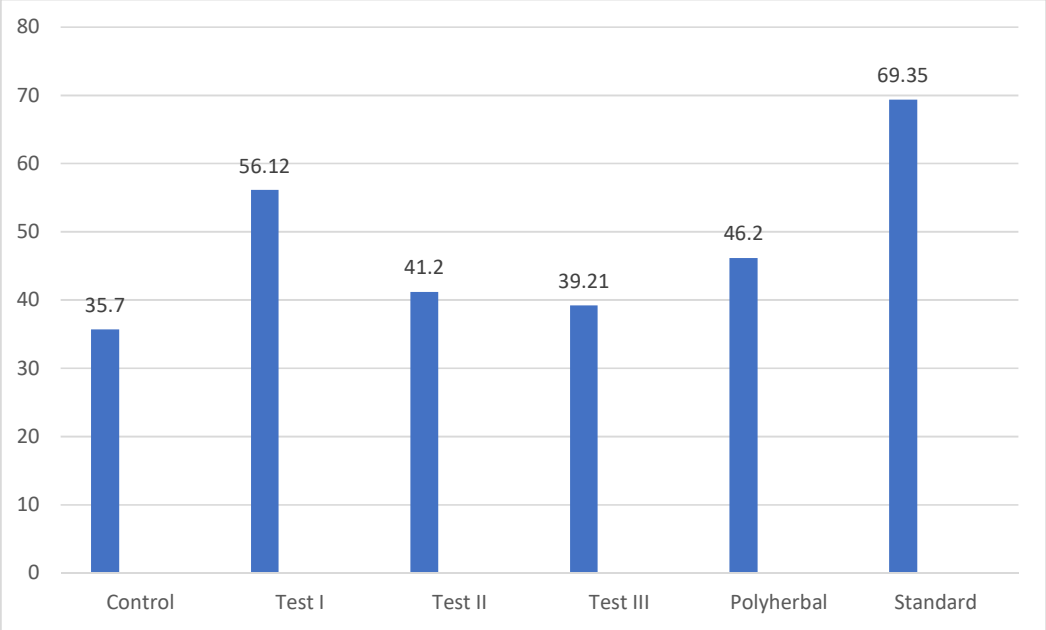
Table3 :Dry,Wet,Hydroxyproline Estimation



Graph 1: Wet granuloma Weight



Graph 2 : Dry granuloma Weight



Graph 3: Effect of extract on Hydroxyproline estimation

Wound Contraction and Epithelization time in Burn wound model

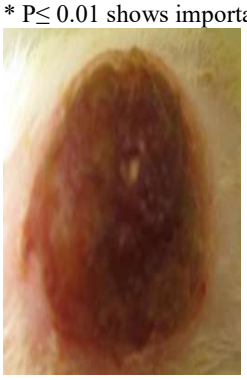
Effect of control, Test I, Test II, Test III, polyherbal formulation, standard drug, was observed on percentage wound contraction in Burn wound model on Initial, 4th, 8th, 12th, 16th day interval which is shown in **Table 4**which indicate that highest wound contraction took place in case of animals treated with Polyherbal Formulation took 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 recovery was seen in control and took 24 days for complete healing and fastest recovery shown by standard drug which is silver sulphadiazine which took 16 days for complete healing**.** Percentage wound contraction in burn wound model shows in **Graph 4, 5**.

	Shrinkage of wound in square millimetre					
Class	**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)	14.11±0.92	39.92±1.21	56.12±0.92	75.12±0.86	85.99±0.72	19
III (Test-II)	11.14±1.22	30.11±1.22	43.94±1.11	61.12±0.66	70.12±1.29	21
IV (Test-III)	13.11±1.92	31.22±2.12	41.12.±32.	56.12±0.98	66.11±0.98	22
V (Polyherbal)	14.12±2.12	40.99±0.98	61.11±0.52	77.12±1.92	88.11±1.13	18

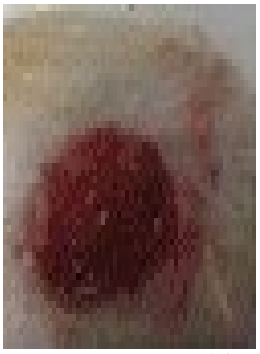
VI (silver sulphadiazine)	12.13±2.12	40.24±1.24	66.12±1.29	91.92±0.92	99.19±0.71	16

Table 4: Percentage wound contraction in Burn wound model

Initial wound area approx. 500 sq mm91.92
≈ n = 6 Rats/Class.
≠ Result shown as Mean Area ± S.E.M
* P≤ 0.01 shows importance when compared to control.



Day01 (G I)

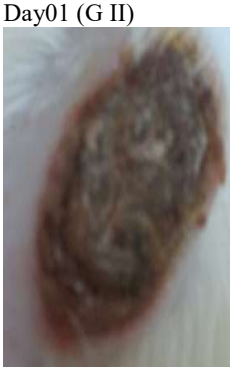


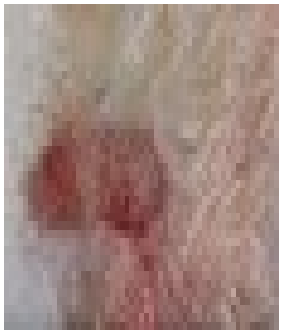
Day16

(G I)

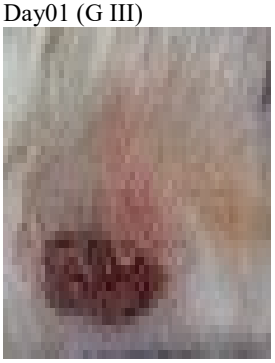
Day01 (G II)

Day16 (G II)

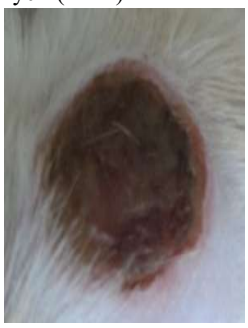




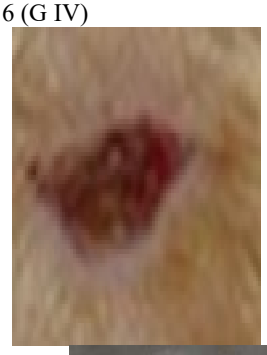
Day01 (G III) Day16 (G III)

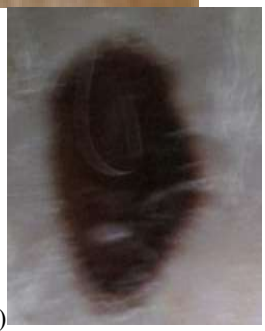


Day01 (G IV)



Day16 (G IV)

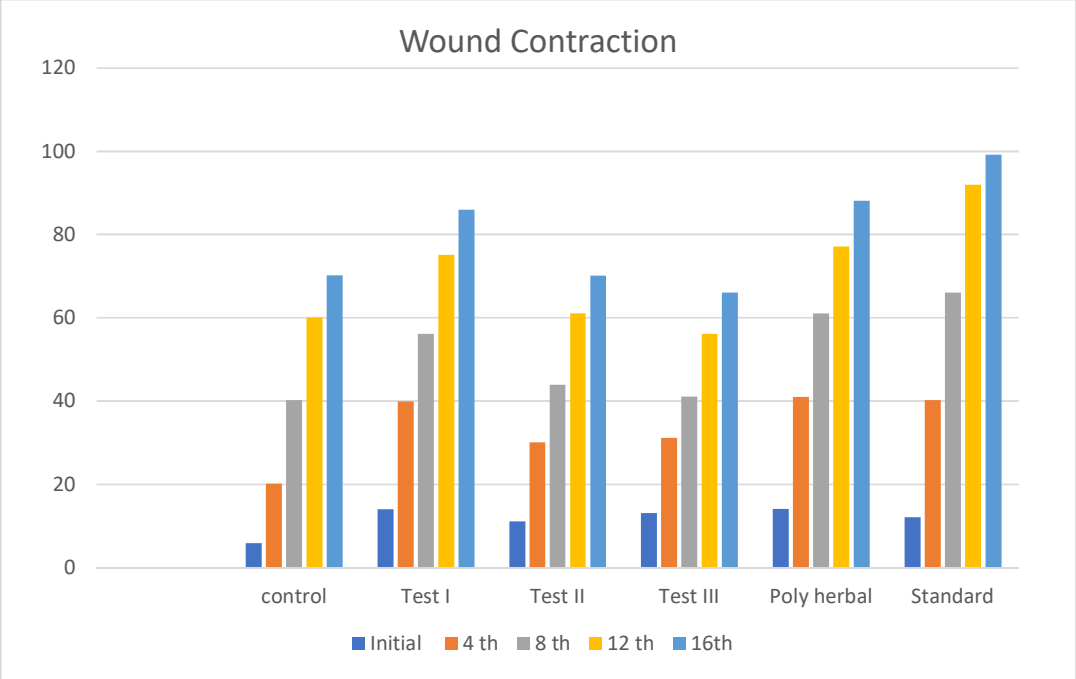




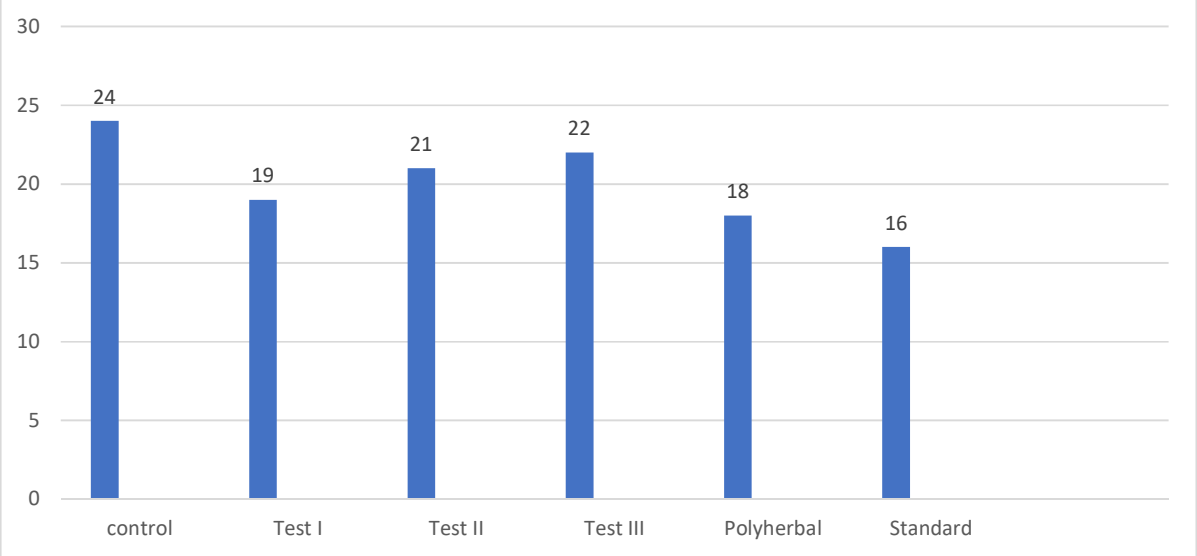
Day01 (G V) Day16 (G V)

Day01 (G VI) Day16 (G VI)

Fig 1: Wound contraction in Burn wound model (Ist and 16th day)



Graph 4: Shrinkage of wound



Graph 5: Epithelization in days

Conclusion; Wound healing evaluation was based on certain parameters like wound contraction, epithelization time, Tensile Strength, Dry and Wet Granuloma Weight, Proline determination

Effect of control, Test I, Test II, Test III, polyherbal formulation, standard drug was observed on granuloma production and proline estimation which shown in **Table3** which indicate that animals received suspension of Polyherbal formulation, Test drug I, Test drug II, Test Drug III, ls shows their Wet and dry granuloma weight, proline Estimation, control group where animals not received any treatment shows least and standard drug received animal shows highest granuloma weight and proline estimation. **Graph 1,2, 3 and table 3** indicate effect of various extract on granuloma weight and on proline estimation

Effect of control, Test I, Test II, Test III, polyherbal formulation, standard drug, was observed on wound contraction in Burn wound model on Initial, 4th, 8th, 12th, 16th day interval which is shown in **Table 4** which indicate that highest wound contraction took place in case of animals treated Polyherbal Formulation took 18 days and Test drug I took 19 days, Test drug II took 21 days, Test drug III took 22 days for complete recovery. The least recovery was seen in control and took 24 days for complete healing and fastest recovery shown by standard drug which is silver sulphadiazine which took 16 days for

complete healing**.** Percentage wound contraction in burn wound model shows in **Graph 4,5** and fig 1.

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