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RESEARCH ARTICLE

Qualitative Evaluation and Wound Healing Potential of Selected Medicinal Plants by Excision Wound Model

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ABSTRACT

Objective: The aim of the paper was to assess the wound healing potential of three medicinal plants using the excision wound healing model on albino rat. **Materials and Methods:** Soxhlet extraction method was utilized for the partition of the constituent of interest. Qualitative analysis and phytochemical screening were performed for the detection of tannins, alkaloids, resins, flavonoids, glycosides, steroids, proteins, carbohydrates, and amino acids. Three plants extract used for the ointment formulation and prepared by the addition of extract of *Artocarpus heterophyllus, Murraya koenigii,* and *Punica granatum* in polyethylene glycol ointment base. Three ointment formulations and six extracts with 5% and 10% extract concentration have been used. Excision wound rat model utilized for the wound healing potential. **Results and Conclusion:** All three plants, including *A. heterophyllus* Lam., *M. koenigii* Linn., and *P. granatum* Linn. extracted for the active constituent. The pharmacological evaluation on the excision wound healing model suggested that Group-I animals showed 52.09% of healing, whereas povidone-iodine treated animals showed 100.00% healing. On the other hand, the ointment formulation treated F-1 showed 96.47% of wound healing, F-2 showed 97.68% healing, and F-3 showed 99.11% healing. The overall healing results can be represented as following: Control <MKL5 <MKL10 <PGB5 <PGB10 <AHP5 <AHP10 <F-1 <F-2 <F-3 <Standard. F3 ointment formulation is better than the F2 and F1 formulation in wound healing potential as compared to others. **Discussion:** These studies have indicated that ointment formulations of *A. heterophyllus, M. koenigii, P. granatum* have been utilized for wound healing potential and it is safer for topical application. Excision wound healing model suggested that the three individual plant extract has shown the wound healing potential, although the prepared ointment formulations F3 have best and synergistic action than the individual. The ointment formulations containing plant extracts in 10% amount have better wound healing potential.

Keywords: Alkaloid, ointment, povidone-iodine, wound healing, wound model

INTRODUCTION

Wound is a characteristic breakdown of anatomic and cellular permanence of cell as well as tissue and damages occur by physical and chemical processes. The poor hygienic condition is a reason for developing wound infection and is the most threaded disease in the developing country.[1,2] Breaking of skin border and opening of the skin is types of physical injuries that cause a wound. Wound healing involves coagulation, matrix formation, remodeling of connective tissue, collagenization, and acquisition of wound strength.[3,4] Many artificial antioxidants have been utilized for wound healing but produce toxic substances, which shall harm human health. Higher ingestion of natural resources such as vegetables and fruits has been proven to reduce the risk[5,6]

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and contains phytochemicals such as carotenoids, alkaloids, vitamins, minerals, and polyphenols[7] those proves beneficiary effects of antioxidant and utilized for wound healing potential.

Hard-to-heal wounds become chronic when the wound is associated with the condition such as vascular disease, diabetes, neuropathy, liver diseases, and hyperglycemia. This type of cases is more in the USA that affects 6.5 million people and for their treatment cost over 25 billion/year.[8] There is need of time to search new therapy modules from natural sources, that minimize therapy, reduce duration of the treatment and also reduce the side effects. Nowadays, NSAIDs is utilized for wound healing potential but they have various side effects i.e. skin irritation, tanning etc. So now, there is time demands that new agents from the natural sources has been extract out as new remedy for the wound healing potential.[9,10]

Nowadays antibiotics and antiseptic are widely used for the wound healing agents by local and systemic delivery. Some topical delivery (benzoyl peroxide gel and ointment) is also utilized for the cure of wounds. From time immemorial, plants are used as medicine and plant-based medicine has been used to cure the diseases and deal with a health problem. The World Health Organization has estimated that 80% of the population today also depends on the natural resources and their traditional knowledge for health beneficiary and health care needs.[11,12]

The aim of the research paper is the assessment of wound heal potential of three medicinal plants includes *Artocarpus heterophyllus* Linn*., Murraya koenigii* Linn*.,* and *Punica granatum* Linn*.*

MATERIALS AND METHODS

Plant material collection and authentication

The pulp of *A. heterophyllus Linn*, plant of *M. koenigii Linn*., and bark of *P. granatum Linn*. were procured from Vidisha district, Madhya Pradesh. The plant samples were given and recognized as pulp of *A. heterophyllus*, bark of *P. granatum*, and plant of *M. koenigii* and authenticated by Dr. Zia ul Hassan, Department of Botany, Saifia Science College, Bhopal. The appession no. for the specimen is 498/BS/saifia/NI_MK_PG_AH/04/16/07 has been preserved for future recognition.

Extraction

The pulp of AH, bark of PGB, and leaves of MKL were separated from the fresh and dried on filter paper sheets under the shade at room temperature until with changing of color of filter papers. The shade-dried, coarsely powdered materials (300 g) were defatted by petroleum ether (43°C). The obtained mass was utilized for the Soxhlet extraction process with seventy percent ethanol. The hydroalcoholic extracts were dried under reduced pressure and room temperature and different extracts were stored at ambient temperature under a closed compartment.

Qualitative test analysis

The constituents in the extracts were identified by following phytochemical screening includes detection of tannins, resins, carbohydrates, flavonoids, alkaloids, steroids, proteins, glycosides, and amino acids.[13-17]

Determination of carbohydrates

Molisch's test: 2–3 ml extract + few drops of α-naphthol solution (20% in ethyl alcohol) + 1 ml conc. H_2SO_4 added. The formation of a violet ring at the junction of two liquid surface indicates the presence of carbohydrates.

Fehling's test: Extract heated with dil. HCl + NaOH + Fehling's solution A and B brick-red precipitate was formed.

Benedict's test: Extract + equal volume of Benedict's reagent. Heat for 3 min solution appears green, yellow, or red.

Determination of proteins and amino-acids

Biuret test: 3 ml of extract + 4% NaOH + 2–3 drops of $CuSO_4$ (1%) solution. Biuret test: 3 ml of extract + 4% NaOH + 2–3 drops of $CuSO_4$ (1%) solution. The red colour shown the presence of amino acid.

Millon's test: 3 ml of extract + Millon's reagent shows the presence of red coloration.

Xanthoproteic test: Extract + conc. nitric acid shown a yellowish-orange color.
Ninhydrin test: Extract + ninhydrin reagent in boiling water bath for 10 min shown violet color appeared.

Determination of steroids

Liebermann–Burchard test: 2 ml extract + chloroform + 1–2 ml acetic acid + 2 drops H_2SO_4, presence of red, after some time blue and in the last appearance of green color.
Salkowski reaction: 2 ml of extract + 2 ml chloroform + 2 ml conc. H_2SO_4. Shake the solution. Red color was shown in chloroform and acid layer shows greenish fluorescence.[4]

Determination of glycosides

Raymond's test: Test solution + 1 ml of 30% ethanol + 0.1% solution of $C_6H_5(NO)_2$ in ethanol + 2–3 drops of NaOH solution (20%) – appearance of violet color, which changes into violet.
Keller–Killani test: 2 ml of extract + glacial acetic acid + one drop of 3% $FeCl_3$ + conc. H_2SO_4. Reddish-brown color at the junction of two-layer and upper layer appeared bluish-green.
Foam test: Test solution containing extract is vigorously shaken with water formation of foam.

Determination of flavonoids and phenolic compounds

Lead acetate test: Filter paper strip was dipped in the alcoholic solution of extract. Ammoniated with ammonia solution – color changed from white to orange.
Shinoda test: Extract + 3 ml 93% alcohol + 0.5 ml of conc. HCl + 0.3 g magnesium turning – pink color observed.

Determination of alkaloids

Mayer's test: Test solution + Mayer reagent – white or yellow precipitate.
Dragendorff's test: Test solution + Dragendorff's reagent showed orange-red precipitate
Wagner's test: Test solution + Wagner's reagent – Brown or reddish-brown precipitate.
Hager's test: Test solution + Hager's reagent – gives characteristic crystalline ppt.

Determination of volatile oil

Dye test: Section + Sudan red III – occurrence of red color

Determination of tannins

Vanillin- HCl test: Extract+ vanillin-HCl reagent – formation of pink or red color.
Gelatin test: Extract solution + aqueous solution of gelatin show white buff color precipitate preparation of ointment formulations.
The hydroalcoholic extracts of three plant have been taken and ointment formulation was formulated. An ointment with a water-soluble base was of the first choice due to their ease of preparation and also eases of cleaning after application.

Ointment formulation

Polyethylene glycol (PEG) base contains the combination of PEG 600 and 4000 (7:3) for ointment formulation with 10% (w/w) concentration of plant extracts. The ointment formulations were prepared by the Fusion method.[18] The prepared formulations were evaluated by various parameters, for example, consistency and stability.[19-21] The extract used for the ointment formulations was depicted in Table 1.

Pharmacological evaluation

Wound healing activity

Excision wound model was selected for wound healing potential. The models were based

Table 1: Ointment formulation based on three plant extract

S. no.	Content	Formulation code
1.	*Murraya koenigii* whole plant extract (MKL) (10%)+Ointment base	F1
2.	*Punica granatum* bark extract (10%) (PGB)+Ointment base	F2
3.	*Artocarpus heterophyllus* pulp extract (10%) (AH)+Ointment base	F3

on the assessment of the rate of contraction and time required for full epithelization and after the permission, for animal studies from Institutional Animals Ethics Committee (Reg. No. CPCSEA/1413/PO/ES/07) albino rats were procured and rats of either sex weighing 130–200 g were selected, maintained at 24–28°C, free access to food and water, and kept in well-ventilated with the alternate dark-light cycle of 12 h throughout the studies.

Excision wound model

In the excision wound model, 72 albino rats were taken divided in 12 groups of six each. Rats depilated by removing hairs at the dorsal thoracic region before wounding. Rats were anesthetized by xylocaine jel I.P. (2% w/v) before excision circular wound of about 2.3 cm diameter under aseptic conditions and were observed throughout the study.[22,23]

To perform the experiment, the rats were divided into 12 groups ($n = 6$).

Group I: Kept as the control group which received simple vehicle (PEG ointment base); Group II: Standard group received povidone-iodine; Group III: Kept as Test I which received formulation F1; Group IV: Kept as Test II which received formulation F2; Group V: Kept as Test III which received formulation F3; Group VI: Test group which received MK 3% extract topically; Group VII: Test group which received MK 10% extract topically; Group VIII: Test group which received PG 3% extract topically; Group IX: Test group which received PG 10% extract topically; Group X: Test group which received AH 3% extract topically; Group XI: Test group which received AH 10% extract topically; and Group XII: Kept as control Group II which received simple vehicle.

Starting from 1st day of wounding, all the samples were applied once daily for 16 days. The 4th, 8th, 12th, and 16th denoted as post wounding days, percentage wound closure was seen on those days. The tracing paper method utilized for the wound area measurement and trace at intervals of 24–48 h. The wound contraction was calculated as a percentage reduction in wound area with respect to the 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.

Wound contraction

Percentage reduction in wound area with respect to initial wound area was calculated as wound contraction as well as the number of days after wounding required to scar to fall off, leaving denoted as epithelization time, where no raw wound behind.[24]

Wound closure (WC) was calculated as

$$\text{Percentage Wound Closure} = \frac{\left(\begin{array}{l}\text{Initial wound Size} - \\ \text{Wound size at} \\ \text{Specific day}\end{array}\right)}{\text{Initial Wound SIze}} \text{x100}$$

Histopathological studies

The ten percent buffered formalin solution used to collect the skin specimen.

The skin specimens were collected in 10% buffered formalin from rats of the 12 groups. Three-micrometer thick sections of the specimen were sliced and stained with dye (hematoxylin and eosin). The collagen formation, fibroblast proliferation, keratinization, and epithelization evaluated by the light microscope.

Statistical analysis

Statistical analysis was performed using GraphPad Prism Software. Raw data obtained from different wound models are expressed as mean ± standard error of the mean. Values <0.03 were considered to be statistically significant.

RESULTS AND DISCUSSION

Extraction

The extraction was done by the Soxhlet apparatus. The drug-free from the fatty materials procured by the addition of petroleum ether. The obtained marc was then extracted with 70% hydroalcoholic solution. The drying of extract containing solvent

(70% hydroalcoholic solution) was done by a rotary vacuum evaporator. The mass of extract was weigh and yield of the hydroalcoholic extract from different plants was tabulated in Table 2.

Phytochemical screening

Preliminary phytochemical screening was performed for each hydroalcoholic extract of *P. granatum* Linn. (PGB), *Murraya Koenigi* Linn. (MKL), and *A. heterophyllus* Linn. (AHP). The AHP has shown the existence of phenol compounds, flavonoids, carbohydrates, proteins, amino acids, terpenoids, glycosides, alkaloids, and tannins.

Qualitative analysis

Dried extracts were taken for the chemical test for detection of the phytoconstituents such as alkaloids, flavonoids, tannins, sterols, phenolic compounds, terpenoids, and carbohydrates. To detect the various constituents present in the different extracts of PGB, MKL, and AHP. Those were subjected to the tests as per identification and chemical test [Table 3].

Pharmacological evaluation

The ointment formulations (F1, F2, and F3) and six extracts with 5% and 10% concentration were evaluated for the wound healing probability as well as for the anti-inflammatory potential.

Wound healing activity

Excision wound model

The percentage of wound contraction ranged from 17.81% to 26.38% in the period from 4 to 8 days and from 39.07% to 53.20% in the period from 12 to 16 days in the control (ointment base) group of *albino* rats, whereas whole epithelization and healing were shown on day 25. The shedding of Eschar lasted for an average of 25.27 days without leaving any residual raw wound in the control *albino* rats. The percentage of wound contraction in *albino* rats treated externally with F1 ranged from 49.94% to 65.76% in the period from 4 to 8 days

Table 2: Percentage yield of hydroalcoholic extracts from different plants

S. no.	Parts	Extract color	Yield (in g)
1	AHP	Yellowish	8.24
2	PGB	Dark brown	8.97
3	MKL	Greenish-brown	20.76

Initially, 80 g of crude drug was taken. Where AHP: Hydroalcoholic extract of *Artocarpus heterophyllus* pulp, PGB: Hydroalcoholic Extract of *Punica granatum Linn*. Bark and MKL: Hydroalcoholic Extract of *Murraya koenigi* Linn. whole plant

Table 3: Qualitative analysis of an extract of *Punica granatum* Linn, *Murraya koenigi*, and *Artocarpus pulp*

S. no.	Chemical test	PG	MK	AH
1.	Carbohydrate			
a.	Molisch test	+	+	+
b.	Fehling test	+	+	+
c.	Benedicts test	+	+	−
2.	Protein			
a.	Biuret test	+	+	−
b.	Millon's test	+	−	+
c.	Xanthoproteic test	+	+	+
3.	Amino acid			
a.	Ninhydrin test	+	+	+
4.	fats and oils			
a.	Filter paper test	−	−	−
5.	Steroid (Tri-terpenoid)			
a.	Salkowski reaction	−	+	+
b.	Liebermann–Burchard reaction	−	+	+
6.	Glycosides			
a.	Raymonds test	+	+	+
b.	Keller–Killani test	+	+	+
c.	Foam test	+	+	+
7	Flavonoids and phenolic compound			
a.	Shinoda test	+	+	+
b.	Lead acetate test	+	+	+
c.	5% $FeCl_3$ solution	+	+	+
7.	Alkaloids			
a.	Dragendorff's test	+	+	+
b.	Mayer's test	+	+	+
c.	Wagner's test	+	+	+
d.	Hager's test	+	+	+
8.	Phenolic compounds			
a.	5% $FeCl_3$ solution	+	+	+
b.	Lead acetate test	+	+	+
9.	Volatile oil			
a.	Sudan red	−	−	−
10.	Tannins			
a.	Vanillin HCl	+	−	+
b.	Gelatin	+	−	+

(−ve = Absent and +ve =Present)

and from 82.78% to 96.47% in the period from 12 to 16 days, respectively. The percentage of wound contraction in *albino* rats treated externally with F-2 ranged from 55.46% to 72.15% in the period from 4 to 8 days and from 86.17% to 97.68% in the period from 12 to 16 days, respectively. The percentage of wound contraction in albino rats treated externally with F3 ranged from 56.30% to 72.15% in the period from 4 to 8 days and from 86.17% to 97.68% in the period from 12 to 16 days, respectively [Table 4 and Figure 1].

The standard (povidone-iodine) treated *albino* rats showed increased wound contraction from 32.89% to 58.07% in the period from 4 to 8 days and from 93.53% to 100% in the period from 12 to 16 days, respectively. The % rate of wound contraction in *albino* rats treated externally with MKL5 ranged from 18.76% to 47.05% in the period from 4 to 8 days and from 62.69% to 84.35% in the period from 12 to 16 days, respectively. The excision wound healing activity of the control group was shown in Figure 2. The percentage of wound contraction in *albino* rats treated externally with MKL10 ranged from 27.87% to 53.13% in the period from 4 to 8 days and from 71.62% to 90.79% in the period from 12 to 16 days, respectively [Table 4 and Figure 1].

The percentage of wound contraction in *albino* rats treated externally with PGB5 ranged from 27.31% to 53.48% in the period from 4 to 8 days and from 80.95% to 92.17% in the period from 12 to 16 days, respectively. The excision wound healing activity of the standard group was shown in Figure 3. The percentage of wound contraction in *albino* rats treated externally with PGB10 ranged from 26.15% to 57.13% in the period from 4 to

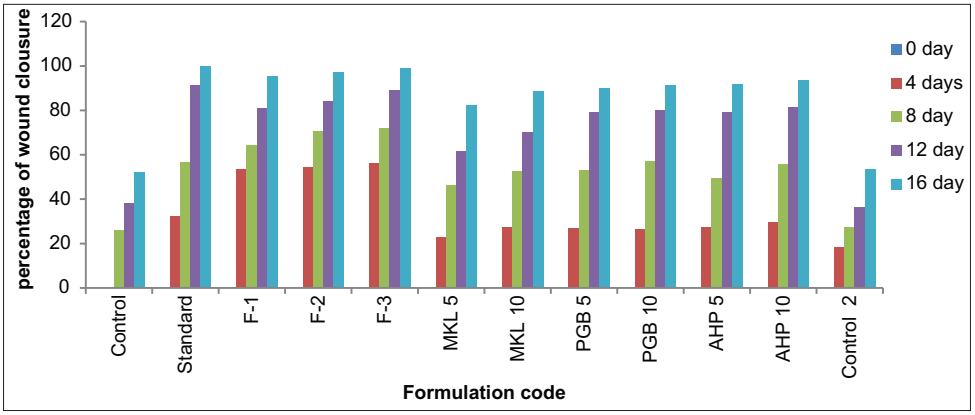

Figure 1: Percentage of wound closure in animal groups at different days

Table 4: Percentage of wound closure in animal groups at different days

Group no.	Groups (*n*)	1st day	4th day	8th day	12th day	16th day
I	Control	0.00	17.81	26.38	39.07	53.20
II	Standard	0.00	32.89***	58.07***	93.53***	100***
III	F-1	0.00	49.94**	65.76**	82.78**	96.47**
IV	F-2	0.00	55.46*	72.15*	86.17**	97.68*
V	F-3	0.00	56.30***	71.65***	88.93***	99.11***
VI	MKL5	0.00	18.76	47.05	62.69	84.35
VII	MKL10	0.00	27.87	53.13	71.62	90.79
VIII	PGB5	0.00	27.31	53.48	80.95	92.17
IX	PGB10	0.00	26.15	57.13	79.99	91.37
X	AHP5	0.00	27.32	49.26	79.02	91.76
XI	AHP10	0.00	23.80	53.03	81.40	94.93
XII	Control 2	0.00	18.57	27.86	36.34	54.62

n=6 animals in each group, values are expressed as Mean ±SEM, If *=*P*<0.05, ** *P*<0.01, *** *P*<0.001, when compare to control. Group I: Control-I, Group II: Standard, Group III: F-1; Group IV: F-2; Group V: F-3; Group VI: MKL5; Group VII: MKL10; Group VIII: PGB5; Group VIX: PGB10; Group X: AHP5; Group XI: AHP10; Group XII: Control 2, whereas F1 (*Murraya koenigii* extract (10%)+Ointment base); F2 (*Punica granatum* extract (10%)+Ointment base); F3 (*Artocarpus heterophyllus* extract (10%)+Ointment base), *M. koenigii* Linn. extract (MKL), *P. granatum* Linn. extract (PGB). and *A. heterophyllus* Lam. extract (AHP)

8 days and from 79.99% to 91.37% in the period from 12 to 16 days, respectively.

Photographs of animals skin

The percentage of wound contraction in *albino* rats treated externally with AHP5 ranged from 27.32% to 49.26% in the period from 4 to 8 days and from 79.02% to 91.76% in the period from 12 to 16 days, respectively. The excision wound healing activity of the test-I group (F-1) was shown in Figure 4. The percentage rate of wound contraction in *albino* rats treated externally with AHP10 ranged from 23.80% to 53.03% in the period from 4 to 8 days and from 81.40% to 94.93% in the period from 12 to 16 days, respectively.

Meanwhile, the mean epithelialization time decreased from 25.27 days in controls to 14.20 days for standard, 17.36 days for F1, 16.8 days for F-2, 15.1 days for F-3, 22.31 days for MKL5, 22.15 days for MKL10, 21.24 days for PGB5, 20.79 days for PGB10, 20.65 days for AHP5, and 19.04 days for AHP10, while standard povidone-iodine showed the lowest time of 14.20 days. Excision wound healing activity of test-I group (F-2) was shown in Figure 5. The overall epithelialization time can be presented as: MKL5 >Control >MKL10 >PGB5 >PGB10 >AHP5 >AHP10 >F1 >F2 >Standard. Among the prepared ointment formulations, the blended formulation showed the increase in the percentage of wound contraction and diminished

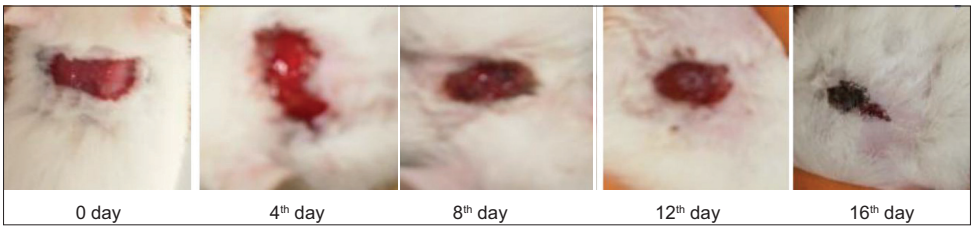

Figure 2: Excision wound healing activity of control group

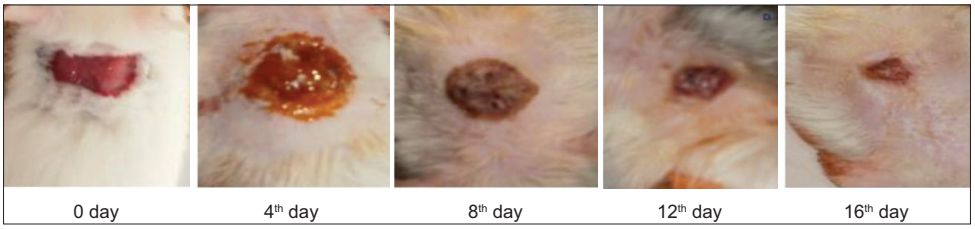

Figure 3: Excision wound healing activity of a standard group

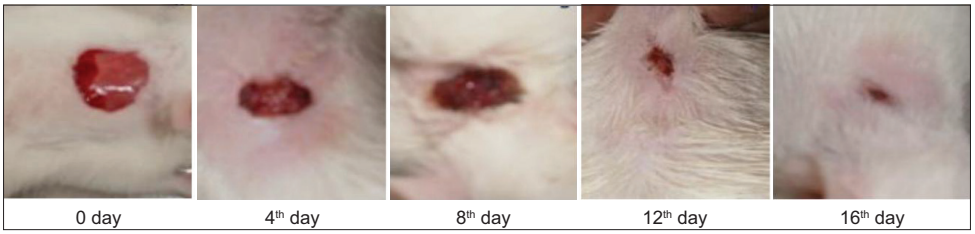

Figure 4: Excision wound healing activity of test-I group (F-1)

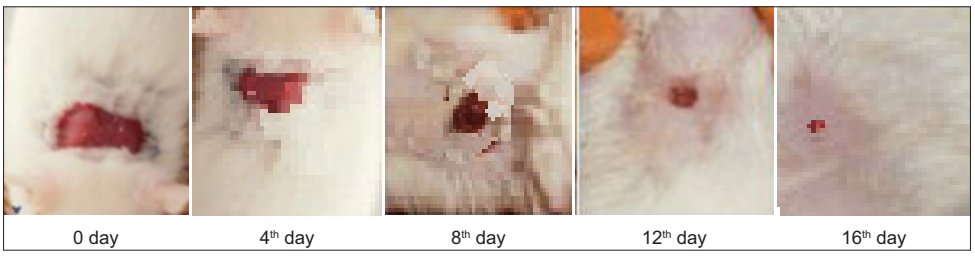

Figure 5: Excision wound healing potential of test-II group (F-2)

in epithelization period in comparison with controls, individual extracts, and as compared to the ointment formulation [Table 4].

The excision wound healing model expresses that 12 groups have shown a reduction in wound area from day 1 to day 16. In spite of that, post wounding day 16, Group-I animals showed 52.09% of healing, whereas povidone-iodine treated animals have shown 100.00% healing. On the other hand, the ointment formulation treated F-1 showed 96.47% of wound healing, F-2 showed 97.68% healing, and F-3 showed 99.11% healing. The individual drug extracts at selected different concentrations also shown wound healing properties as per the concentration. The MKL5 and MKL10 have shown 84.35% and 90.79% healing. The wound healing activity of test-I group (F-3) was shown in Figure 6. The PGB5 and PGB10 have shown 92.17% and 91.37% healing. The AHP5 and AHP10 have shown 91.76% and 94.93% healing. All readings are found to be statistically noteworthy and comparable with control. The overall healing results can be represented as following: Control <MKL5 <MKL10 <PGB5 <PGB10 <AHP5 <AHP10 <F-1 <F-2 <F-3 <Standard [Table 4 and Figure 1].

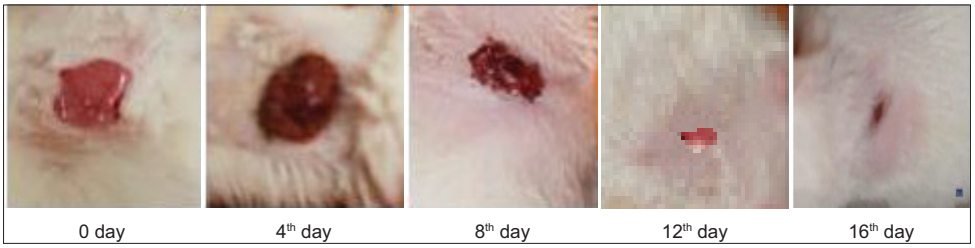

Figure 6: Excision wound healing activity of test-III group (F-3)

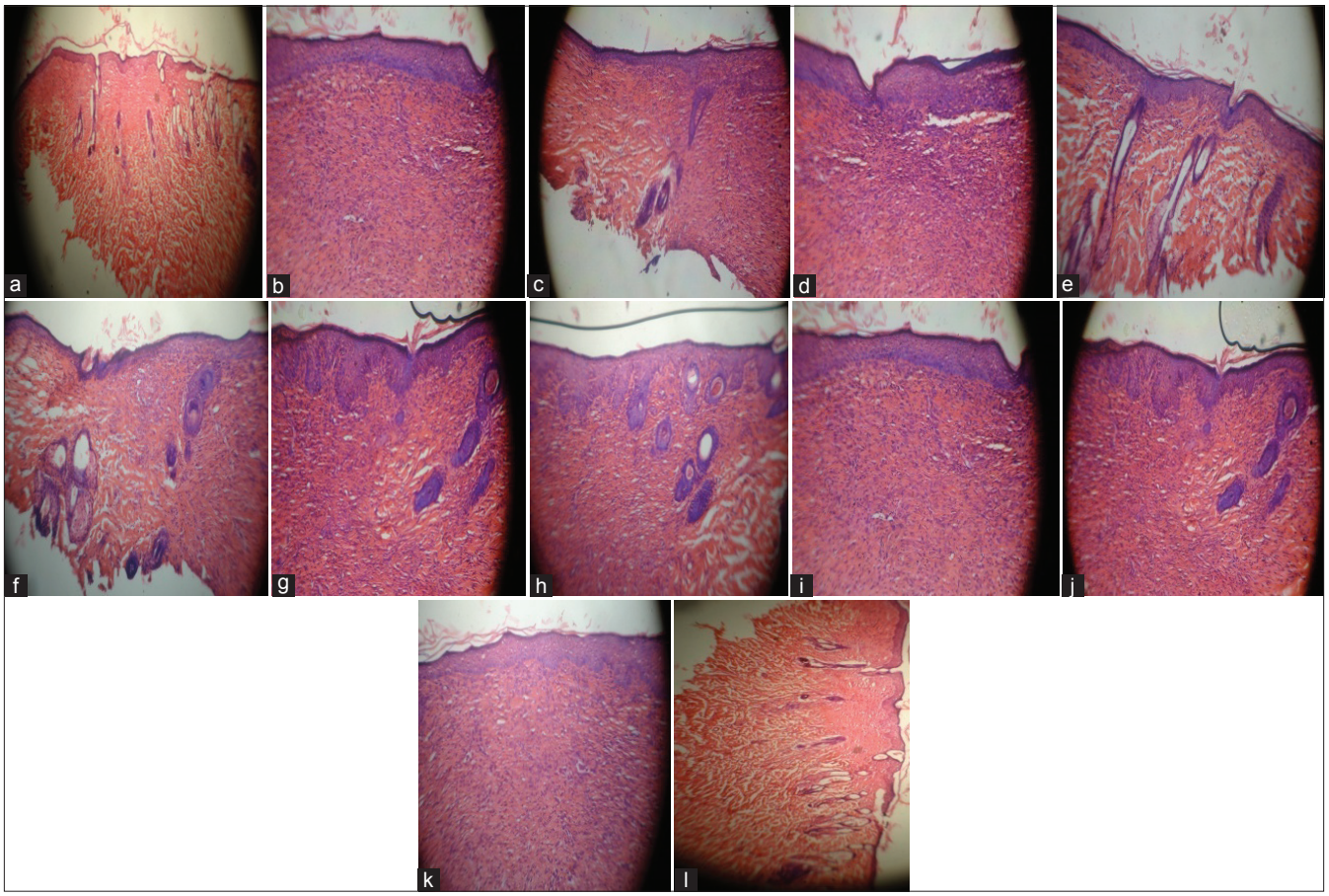

Figure 7: (a-l) Histopathology of skin at day 16 stained with hematoxylin-eosin (×100). (a) Group-I, (b) Group-II, (c) Group-III, (d) Group-IV, (e) Group-V, (f) Group-I, (g) Group-I, (h) Group-I, (i) Group-I, (j) Group-I, (k) Group-I, (l) Group-I

Histopathological changes (excision)

The biopsy at the healed day had shown the normal epithelial which evident the healing of the skin structures and process of epithelialization. It was also observed that adnexa was restored and fibrosis was reinstated within the dermal tissue in ointment formulations and standard treated groups. The control groups had shown a delayed effect in contrast to the treated groups in the context of ground substance formations in the granulation tissues. Histopathology of skin at day 16 stained with hematoxylin and eosin was shown in Figure 7a-l. The presence of scattered fibroblasts and mononuclear inflammatory cells has shown minimal fibrosis within the granulation tissues of the control groups indicating the lower intensity of healing phenomena. This was also supported by the observation, for example, the presence of lesser propagation of vasculature in granulation tissue. The treatment groups of ointment formulations and the standard treated groups showed the presence of eosinophilic collagen tissues in an extensive amount in the tissue of granulations. The healing of tissues by fibrosis was also adjudged by the neovascularization observed with the presence of lesser inflammatory cells in the granulation tissues. The order of healing can be arranged as follows: Control 1< control 2 <MKL5 <MKL10 <PGB5 <PGB10 <AHP5 <AHP10 <F-1 < F-2 <F-3 <Standard. These results were similar to that which was observed for wound contraction and rate of epithelization.

CONCLUSION

All plant extract was evaluated for the various constituents present and phytochemical screening was performed for the detection of tannins, alkaloids, resins, glycosides, flavonoids, steroids, proteins, carbohydrates, and amino acids. The extracts prepared using the extraction method were incorporated in the ointment base for ointment formulation. Excision wound model suggested that the three individual plant extract has shown the wound healing potential although the prepared ointment formulations F3 have better and synergistic action than the individual. The ointment formulations containing plant extracts in 10% amount have better-wound healing potential. The order of healing can be arranged as follows: Control 1< control 2 <MKL5 <MKL10 <PGB5 <PGB10 <AHP5 <AHP10 <F-1 <F-2 <F-3 <Standard. The result of pharmacological evaluation suggested that ointment formulation (F3), provides a better remedy for the management of wounds. The ointment formulations were prepared from the hydroalcoholic extract of *M. koenigii*, *P. granatum,* and *Artocarpus heterophyllus* has shown a decrease in wound compared to the control group. The ointment formulation provides a better pharmacological activity, so it is useful for the treatment of wound and also helpful to early improvement in wound healing and reduce the side effect as well.

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