Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2012, 2 (3):355-363

ISSN : 2249-7412
CODEN (USA): AJPSKY

Wound healing activity of topical application form based on herbomineral formulation

Sushil S. Pimpare, Yogesh T. Sonawane, Chetan A. Chaudhari, Lalit P. Sali, Naveenkumar P. Jain and Chhaya H. Gadgoli*

Department of Pharmacognosy, Saraswathi Vidya Bhavan's College of Pharmacy, Sonarpada, Dombivli (E), Thane, Maharashtra, India

---

ABSTRACT

*The traditional **In**dian medic**in**e—Ayurveda, describes various herbs, fats, oils and m**in**erals with anti-ag**in**g as well as wound heal**in**g properties. Mimusops elengi (Sapotaceae) commonly called as 'Bakul' in India. In Ayurveda, M. elengi has been reported to be used for arresting bleeding of gums and preparation of a lotion for sores and wounds. Zinc is a trace mineral, a component of many enzymes, including DNA and RNA polymerases, Matrix metalloproteinase ((MMPs) and is also required for protein synthesis, DNA synthesis, mitosis, and cell proliferation. Many of these zinc-dependent processes are required for wound healing, such as collagen synthesis and cell division. Consequently, zinc forms one of essential nutrients for normal wound healing. In the present study Yashad Bhasma is used as a source of zinc. There was no scientific evidence justifying the use of Mimusops elengi bark along with Yashad Bhasma for treatment of wound, therefore the present study was aimed at evaluation of wound healing activity of the plant (Bark) in combination with Yashad Bhasma in Excision, Incision and Estimation of biochemical marker models.*

Key Word index: Zinc, Yashad Bhasma, *Mimusops elengi*, *Sapotaceae*, Wound Healing.

INTRODUCTION

The skin is the largest organ of the body with the purpose to serve as barrier against external agents. Wound is a clinical entity and is as old as mankind, often possesses problem in clinical practice. A lot of research has been envisaged to develop the better healing agents and it has been a challenging task to discover healing agents and keep up pace with problems encountered [Schwartz S I, Schwartz's Principles of Surgery, The McGraw-Hill Publishing House, New York, 2006, 1-5].

Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue. Wounds can be broadly categorized as having either an acute or a chronic etiology including bites, burns, surgical wound abrasion, laceration or acute inflammatory phase followed by synthesis of collagen and the extracellular macromolecules which are later remolded to form, scar [Deodhar A K, Rana R E, J Postgrad Med, 1997, 43, 52–56].

The inflammatory phase begins immediately after injury, first with vasoconstriction that favors homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformulations and improvement in the components of the collagen fiber that increases the tensile strength [Mandelbaum S H, Di Santis E P, Mandelbaum M H S A, Anais Brasileiros de Dermatologia, 2003, 78, 393–410].

Healing requires the collaborative efforts of many different tissues and cell lineages. It involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization.

Healing is not complete until the disrupted surfaces are firmly knit by collagen [Govindrajan R, Kumar B, Vijaykumar M, Pushpangadan P, J Ethnopharmaco, 2007, 114, 103–113]. Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns [Govindrajan R, Kumar B, Vijaykumar M, Pushpangadan P, J Ethnopharmaco, 2007, 114, 103–113]..

Mimusops elengi L. (Sapotaceae) is a large glabrous evergreen tree found in the Western Peninsula of India. The tree is planted throughout India for its ornamental foliage and the fragrance of its flowers. Its bark is acrid, sweet, cardiotonic, alexipharmic, stomachic, anthelmintic, astringent, and is also used to cure biliousness and diseases of the gums and teeth [Akhtar N, Ali M, Alam S, Nat Prodt Res, 2010, 24, 962-972]. Bark has exhibited Anti gastric ulcer activity [Shah P J, Gandhi M S, Shah M B, Goswami S S , J Ethnopharmaco, 2003, 89, 305-311], antimicrobial [Ali M A, Mozid M A, Yeasmin M S, Khan A M and Sayeed M A, Res J Agri and Bio Sci, 2008, 4, 871-874], Antibiotic [Rangama B N L D, Abayasekara C L and Panagoda G J, M.R.D.M. Senanayake, J.Natn.Sci.Foundation Sri Lanhka, 2009, 37, 139-145], In vitro Anthelmintic [Mali R G, Mahajan S G and Mehta A A, Pharmacog mag, 2007, 3, 73-76] and Diuretic activity [Koti B, Ashok P, Int J Green phar, 2010, 4, 90-92].

The bark has been reported to contain Volatile oil [Ruikar A ,Torane R ,Tambe A ,Puranik V,Deshpande N, Int J Chem Tech Res, 2009,2, 158-161], Taraxerone, Taraxerol, α- spinasterol, ursolic acid, Betulinic acid, β-D-glucoside of β-sitosterol [Misra G and Mitra C R, Phytochemistry, 1967, 6, 1309] and Gallic acid esters [Akhtar N, Ali M, Alam S, Nat Prodt Res, 2010, 24, 962-972]. The plant is found to be very useful in traditional systems of medicines like Ayurveda.

In Ayurveda, bark of *M. elengi* Linn finds its utility in arresting bleeding of gums and skin disorders, however, the literature survey revealed that no scientific study has been carried out on wound healing activity. Bhasma are Ayurvedic preparations, in which pure metal is treated with certain Ayurvedic decoctions and then treated to high temperature to burn the metal in a closed cupola/crucible. The mineral ash that remains behind is called “Bhasma” of that metal. Calcinated zinc oxide containing chiefly zinc oxide is known for its mild astringent and antiseptic properties, due to which wound healing is accelerated.

An Ayurvedic preparation containing zinc in the form of Calcinated zinc oxide is utilized in the present study to evaluate efficacy of the bhasma in combination with the extract. In the present study, an effort has been made to establish wound healing potential of the methanolic extract of the bark in combination with Yashad bhasma, using different models.

MATERIALS AND METHODS

2.1. Materials

Bark of the plant *M. elengi* was procured from the local market, Mumbai. The bark was authenticated at Agharkar Research Institute, Pune. by carrying out macroscopic and microscopic evaluation. The voucher specimen of the same has been deposited (voucher specimen no. S/B 086).

2.2. Preparation of the extract

The air-dried crude drug was pulverized to obtain coarse powder. The powdered drug (150gm) was defatted by extracting with pet-ether (60–80 ◦C) using Soxhlet Extractor. The defatting process was followed by extraction of the powdered drug with methanol with Soxhlet extractor. The extracts thus obtained were concentrated by recovering the solvent by Rotary Flash Evaporator . The concentrated extract was then evaporated to dryness in

vacuum oven at temperature not more than 50 ^{0}C (yield: 29.98% W/W). The dried extract was stored at 2–8 ˚C in refrigerator. The extract was further used for the evaluation of wound healing activity.

2.3. Phytochemical analysis
The methanolic extract was tested qualitatively for different Phytoconstituents by performing various qualitative chemical tests. The content of total phenols in the extract was determined using Folin-Ciocalteu method [Yu L, Perret J, Harris M, Wilson J, Haley S, J Agri and Food Chem 2003, 51, 1566–1570] using Gallic acid (Molichem, India) as reference standard.

2.4. HPTLC fingerprinting study
Preliminary phytochemical analysis revealed presence of sterols and triterpenoides. The bark is reported to contain β-sitosterol and Lupeol as steroidal and triterpenoidal compounds respectively. Therefore, the qualitative HPTLC analysis was carried out using HPTLC (Silica gel GF_{254}) plates and Toluene: Ethyl acetate: Methanol in the ratio 15: 3: 1.5 (v/v) and Toulene: Ethyl acetate: Formic acid in the ratio 9: 1: 2 (v/v) as mobile-phase for detection of β-sitosterol and Lupeol respectively. Sample detection was done by UV absorbance at 200nm and detection was enhanced by a spraying with Liebermann Burchard reagent and Anisaldehyde sulphuric acid reagent for β-sitosterol and Lupeol respectively. R_f and λmax of the bands were recorded. Quantitative HPTLC study was also performed for the determining the content of β-sitosterol and Lupeol in the methanolic extract by utilizing the same mobile phase and the stationary phase.

2.5. Animals
The healthy Wistar albino rats of either sex, weighing 150–200 g, were housed under standard environmental conditions of temperature and humidity (25±0.50 ◦C) and 12 h light/dark cycle) were utilized for the studies. The animals were fed with standard pellet diet and water ad libitum. The animal studies were performed in the institute with due permission from Institutional Animal Ethical Committee (registration no. 704/CPCSEA, India dated 25/8/2003).

2.6 Preparation of test ointments
Methanolic extract and Yashad Bhasma were incorporated into Simple Ointment Base BP and the following Formulations were prepared by trituration method:

CT: A Simple Ointment Base B.P. was used as control preparation in the study
STD: Betadine® ointment (Marketed by Win Medicare, Batch Manufacturing number PJ0109, New Delhi) containing Povidone Iodine I .P.-5%w/w was used as standard preparation in the study.
T1: The methanolic extract was incorporated in the concentrations of 0.5% w/w into Simple Ointment Base B.P.
T2: The methanolic extract was incorporated in the concentrations of 1% w/w into Simple Ointment Base B.P.
T3: Yashad bhasma was incorporated in concentration of 1.25% w/w (equivalent to Zn^{+2} 0.95% w/w) in simple ointment base B.P.
T4: Yashad bhasma and the methanolic extract were incorporated in concentration of 0.62% w/w (equivalent to Zn^{+2} 0.47% w/w) and 0.5% w/w into simple ointment base B.P.

These formulations were applied topically on wounds.

2.7 Analysis of yashad bhasma
Yashad bhasma (Baidyanath Co.Batch no.47) and the ointments T5 and T6 were analyzed for the zinc content. The content of zinc was determined using inductively coupled plasma atomic emission spectroscopy from sophisticated analytical instrument facility- IIT, Powai, Mumbai.

2.8. Acute dermal toxicity
The acute dermal toxicity testing of the methanolic extract was carried out by applying the Ointments T2 and T3 on the shaved back of the rats. The OECD guidelines no. 402 (OECD guidelines, 1987) were followed for the study. Animals were observed for the sign of redness and itching for 24 hrs.

2.9. Wound healing activity
The animals were grouped into three major groups viz. control, standard and test with six animals in each group. The control group was treated with simple ointment base B.P. The standard group was treated with Betadine (Win

Medicare containing 5% (w/w) povidone iodine, lot no. PJ0109) ointment. The test groups were treated with ointments with different concentrations of extract viz. 0.5% (w/w), 1% (w/w), yashad bhasma (Batch number 47) (1.25% w/w) and yashad bhasma (0.625%w/w) in combination with the methanolic extract (0.50%w/w) incorporated in simple ointment base, in all the three models. Ointments were applied twice a day.

2.9.1. Excision wound model

The rats were anesthetized by administering ketamine (Neon laboratories ltd, batch number SM20125) (0.5 ml/kg b. w. i.p.). A full thickness of the excision wound of circular area (approx. 500mm^2) and 2mm depth was inflicted on the shaved back of the rats 30 min later, the administration of ketamine injection. The wounding day was considered as day 0. The wounds were treated with topical application of the ointment preparations mentioned above, till the wounds were completely healed. The wounds were monitored and the area of wound was measured on 4, 6, 8, 10, 12, 14, 16 post-wounding days. The mean % wound closure is reported in Table 1. The period of epithelization was calculated as the number of days required for falling of the eschar. [Nayak B S, Anderson M, Pereire P, Fitoterap, 2007, 78, 540–544]. Wound healing rate was expressed as [Muthusamy S K, Kirubanandan S, Sripriya, Sehgal P K, J Surg Res, 2008,144, 94–101]:

$$\% \text{ of wound closure} = \frac{\text{wound area on day 0} - \text{wound area on day n}}{\text{Wound area on day 0}} \times 100$$

Where *n* =number of days 4th, 8th, 12th, 16th and 20th day.

2.9.2. Incision wound model

The rats were anesthetized by administering ketamine (Neon laboratories ltd, batch number SM20125) (0.5 ml/kg b. w. i.p.).Two paravertibral Incision wounds of about 6 cm in length and 2mm in depth were made with sterile scalpel on the shaved back of the rats 30 min later, the administration of ketamine injection. The parted skin was kept together and stitched with black silk at 0.5cm intervals (Fig. 3) using surgical thread (no. 000) and a curved needle (no. 9). The continuous thread on both wound edges were tightened for good closure of the wounds. The wounds of animals in the different groups were treated with topical application of the Ointments as described above, for the period of 10 days. The wounding day was considered as day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day (Fig. 5) and the tensile strength of the skin that is the weight in grams required to break open the wound/ was measured by tensiometer for both the sides on the 10th day reported in Table 2 [Kokane D D, More R Y, Kale M B, Nehete M N, Mehendale P C and Gadgoli C H, J Ethnopharmaco, 2009, 124, 311-315].

Tensile strength was calculated using the following formula [Diwan P V, Reddy B S, Reddy R K K. Naidu V G M, Madhusudhana K, Agwane S B, Ramakrishna S, J Ethnopharmaco, 2008, 115, 249–256]:

$$\text{Tensile strength} = \frac{\text{breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^{22})}$$

The results are presented as mean weight in gms required to break open the wound of both the sides ± S.E.M

2.9.3. Estimation of biochemical marker

Circular excision wound with approximate area of 500mm ^{2} was inflicted using the procedure described in 2.9.1. The wounds were treated with topical application of ointments as described above for 10 days. The scab was removed on 11th post wounding day and dried in oven at 110 ^{o}C. The hydroxyproline content in dried scab was determined by the method described by Bergman and Loxley (1963). Hydroxyproline from the scab was extracted using concentrated Hydrochloric acid followed by reaction between amino group of hydroxyproline with pdimetylaminobenzaldeyde to develop red colour. The red colour thus produced, was measured on Spectrophotometer (Elico-SL 159) at 558nm using the reagent blank The results are presented in Table 3 as mean hydroxyproline content/ 500 mg of scab ± S.E.M

2.10. Statistical analysis

Results obtained from the wound healing experiments have been expressed as mean ± SEM and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA test [Mukherjee P K, Verpoorte R, Suresh B, J Ethnopharmaco, 2000, 70, 315–321].

RESULTS

3.1. Phytochemical analysis

Qualitative phytochemical analysis of methanolic extract of stem bark *M.elengi* revealed presence of saponins and tannins. The quantitative estimation of total polyphenolics in the methanolic extract equivalent to Gallic acid by Folin-Ciocalteu method was found to be 21.36% (w/w).

3.2. HPTLC fingerprinting study

Chromatographic analysis of reference standard of β-sitosterol and Lupeol indicated single spot of with R_f 0.65 and R_f 0.44 **(Fig.1- Fig.4)** which coincides with spots in methanolic extract. UV spectra of the standard and the sample peak were also matching which also confirms the presence of β-sitosterol and Lupeol in methanolic extract. The content of β-sitosterol and Lupeol in the methanolic extract were extrapolated from the calibration curves of respective reference standards.

The methanolic extract was found to contain 0.0142 %w/w and 0.067 %w/w of β-sitosterol and Lupeol respectively.

Table 1 Result table of Phytochemical Analysis of extract and the formulations

Phytochemical analysis	HPTLC fingerprinting study	Analysis of yashad bhasma		
		Sample code	Sample Code	Zn^{+2} (% w/w)
Total polyphenolics 21.36% (w/w)	β-sitosterol: 0.0142 %w/w (R_f 0.65) Lupeol: 0.067 %w/w (R_f 0.44)	A	Yashad bhasma(Baidyanath Batch No.47)	46.53
		B	T3	0.95
		C	T4	0.47

Table 2 Wound healing activity

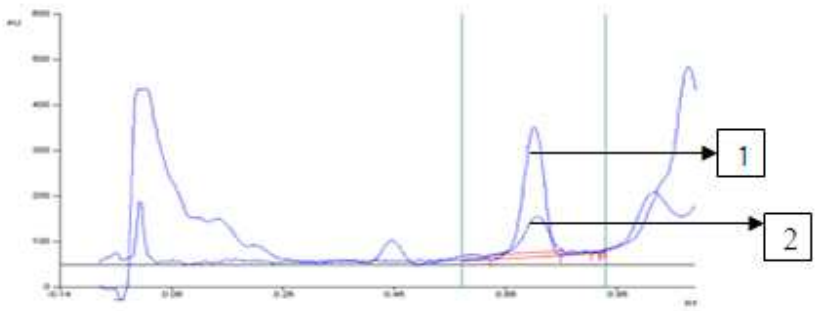
Treatment	% Wound Contraction Day					Epithelization Time (Days)	Hydroxyproline Content (µg/500mg) (Mean ± S.E.M)	Mean Tensile strength in gm (Mean ± S.E.M)
	4th day	8th day	12th Day	16th day	20th day			
CT	10±0.36	35.06±0.38	47.78±0.54	74.51±0.32	91.46±0.23	26	13.41±0.25	387.5±2.14
STD	30.5±0.43	53.48±0.94	83.78±1.1	92.74±0.49	98.01±0.30	22	19.84±0.72	533.33±4.21
T1	12.91±0.32**	55.05±0.86***	82.12±0.63***	88.72±0.22***	94.49±0.13**	22	15.37±1.11*	505±8.85***
T2	16.07±0.64***	67.76±0.43***	86.37±1.38***	95.75±0.41***	100***	20	23.09±0.51***	547.5±7.71***
T3	19.96±0.29***	55.24±0.86***	71.62±0.44***	86.27±0.35***	96.17±0.38***	21	20.69±0.69***	490±10.32***
T4	27.32±0.65***	70.87±0.60***	90.44±1.39***	100***	100***	16	26.78±0.51***	576.67±8.72***

n = 6 Animals in each group

The treated groups are compared by ANOVA test with the control group.

****P<0.001, ** P<0.01, * P<0.05*

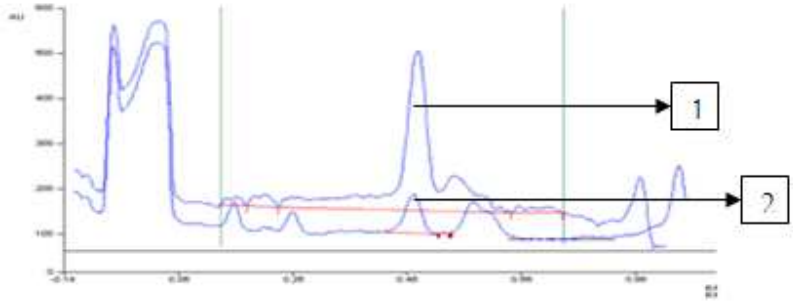
Fig. 1. Chromatograms of Standard β-sitosterol and Methanolic extract of *M. elengi* at 200nm



1 Chromatogram of Standard β-sitosterol (R_f 0.65)

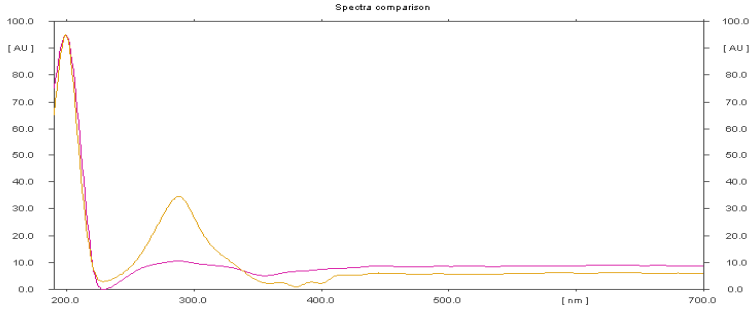
2 Chromatogram of Methanolic extract of M. elengi (R_f 0.66)

Fig.2. Chromatograms of Standard Lupeol and Methanolic extract of *M. elengi* at 200nm



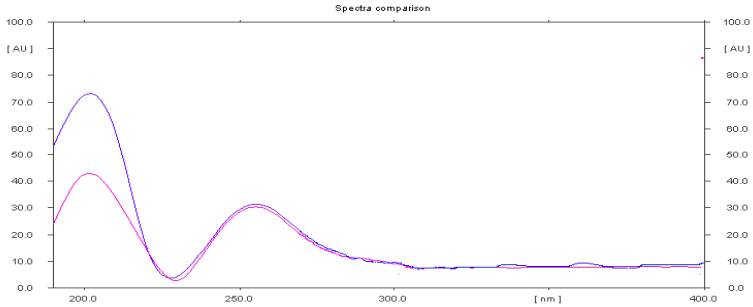
1 Chromatogram of Standard Lupeol (R_f 0.44)
2 Chromatogram of Methanolic extract of M. elengi (R_f 0.44)

Fig.3. Superimposition of the UV spectra of β-sitosterol obtained from standard β-sitosterol and Methanolic extract of bark of *Mimusops elengi Linn.* (λmax 206)



1: UV spectra of Methanolic extract of bark of Mimusops elengi Linn.
2: UV spectra of β-sitosterol obtained from standard β-sitosterol

Fig.4. Superimposition of the UV spectra of Lupeol obtained from standard Lupeol and Methanolic extract of bark of *Mimusops elengi Linn.* (λmax 202)



1: UV spectra of Lupeol obtained from standard Lupeol
2: UV spectra of Methanolic extract of bark of Mimusops elengi Linn.

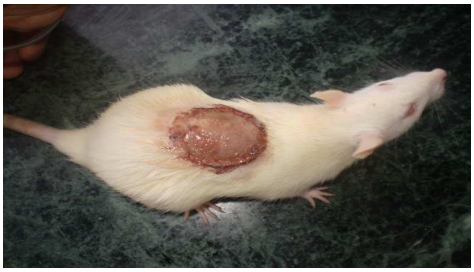


Fig. 5.A circular excision wound on the day 0.

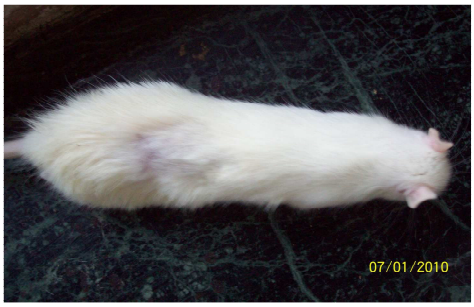


Fig. 6.A circular excision wound after 16 day treatment.

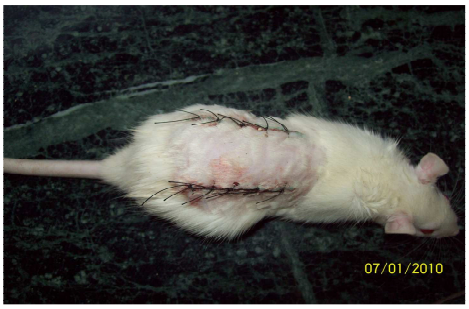


Fig. 7.Incision wound on the day 0.

3.3. Analysis of yashad bhasma
Yashad bhasma (Baidyanath Co.) and the ointment prepared T5 and T6 were analyzed for the zinc content.

3.4. Acute dermal toxicity study
There were no signs of redness, itching, when the formulation T2 and T3 was applied on the shaved back of albino rats. This indicates that there is no observed toxicity and the extracts were found to be safe as no mortality was observed at the highest dose concentration.

3.5. Excision wound study
The results of wound healing activity by excision wound model are presented in **Table2 Fig. 5.** The values presented in the table represent percentage wound healing at 4, 8, 12, 16, 20 days for control (simple ointment B.P. treated group.), standard (povidone iodine treated group) and the test groups viz.T1, T2, T3 and T4. It is observed that maximum wound contraction (100% on day 16) is observed in case of animals treated with ointment T4. The wound contraction or healing was found to be significantly higher ($P < 0.001$) on days 8, 12 and 16 as compared to the control group. Treatment with ointment T4 is found to be the most potent, as the period of epithelization is the least, that is, 16 days **(Fig.6).** The period of epithelization was also found to be significantly ($P<0.001$) low as compared to standard group. The results indicate that formulation is better than the standard drug (Betadine) in wound healing potential.

3.6. Incision wound study

The results of the Incision wound healing studies are presented as mean weight in gram±SEM required to break open the resutured wound **Fig. 7 (Table 2)**. The animals treated with ointment T4 indicated significantly high ($P < 0.001$) tensile strength 576.67 gm as compared to the control group.

3.7. Estimation of Biochemical marker

The results presented in **Table 2** reveal that the animals treated with ointment T4 showed significantly high ($P <0.001$) levels of hydroxyproline (26.78_g/500mg) as compared to the control group (13.41_g/500mg).

DISCUSSION

The basic principle of optimal wound healing is to minimize tissue damage and provide an adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part. The results of excision wound model indicate that in the first 4 days there is no significant increase in the wound contraction in all the groups as compared to the control group. The results of the 8th day indicate that there is significant increase ($P < 0.001$) in the percentage wound contraction in the group treated with standard drug that is povidone iodine, ointment T4, revealing that the extract has ability to induce cellular proliferation, as the second phase of wound healing is proliferative phase. Hydroxyproline is an amino acid which is required for synthesis of protein collagen. Hydroxyproline is a major component of the protein collagen. Hydroxyproline content has been used as an indicator to determine collagen synthesis. The hydroxyproline levels in different groups were proportional to the tensile strength of the healed wounds in those groups. Hydroxyproline is one of the biomarkers indicating wound healing process, as the content of the same is increased on 10th day. The increased hydroxyproline content in the scab of the animals treated with ointment T4 support the wound healing process. Out of these ointments, ointment T4 is found to be the most effective. The increase in tensile strength of wounded skin indicates the promotion of collagen fibers. Highest tensile strength of the wounded skin was observed in the animals treated with ointmentT4. The increased tensile strength reveals that the disrupted surfaces are firmly knit by collagen.

Ointment T4 is found to be the most effective due to presence of zinc in bhasma and presence of β-sitosterol and Lupeol in methanolic extract.

Zinc is a trace mineral, a component of many enzymes, including DNA and RNA polymerases, Matrix metalloproteinases ((MMPs) and is also required for protein synthesis, DNA synthesis, mitosis, and cell proliferation. The MMPs play an important role in tissue remodeling associated with various physiological and pathological processes such as morphogenesis, angiogenesis and tissue repair. Many of these zinc-dependent processes are required for wound healing, such as collagen synthesis and cell division. Consequently, zinc forms one of essential nutrients for normal wound healing [Andrews, Marti, Gallagher-Allred and Charlette, Adv in Wound Care, 1999, 12, 137-138].

Chemically, *M. elengi* Linn bark is a rich source of steroidal and triterpenoidal saponin. The constituents like steroids viz β-sitosterol and triterpenoides viz Lupeol seem to have major role in pharmacological activities. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. In the proliferative phase, angiogenesis is essential for the provision of oxygen and metabolites to tissues. It is already reported that, β-sitosterol has therapeutic angiogenic effect on damaged blood vessels [Choi S, Kim K, Choi J, Han S, Park Y, Lee S, Kim J and Chung M, Planta Medica, 2002, 68, 330-335]. β-sitosterol also exhibited anti-inflammatory, anti-pyretic, antiarthritic and anti-ulcer activities [Patra A, Jha S, Murthy P, Manik and Sharon A, Int J Pharm Sci and Res, 2010, 1, 95-100]. Lupeol shows activities like Antiprotozoal, Anti-inflammatory and Antimicrobial which are also supporting the wound healing process. Lupeol is also used as Nutraceautical/chemopreventive agent [Gallo M B and Sarachine M J, Int J Biomed and Pharmaceu Sci, 2009, 3, 47-66]. The bark has also exhibited antimicrobial [Ali M A, Mozid M A, Yeasmin M S, Khan A M and Sayeed M A, Res J Agri and Bio Sci, 2008, 4, 871-874] and Antibiotic activity [Rangama B N L D, Abayasekara C L and Panagoda G J, M.R.D.M. Senanayake, J.Natn.Sci.Foundation Sri Lanhka, 2009, 37, 139-145] which prevent microbial infection of wound. Phytochemical analysis revealed presence of polyphenolic compounds like tannins and these are also reported to have good wound healing activity [Muthusamy S K, Kirubanandan S, Sripriya, Sehgal P K, J Surg Res, 2008,144, 94–101]. Tannins are known for their astringent activity [Hagermann A E, Tannin Chemistry in Tannin handbook, USA, 2002, 36-42] which may also be responsible for Anti-bacterial activity.

CONCLUSION

The data of this study indicated that the bark extract of *Mimusops elengi* in combination with Yashad Bhasma possesses better wound healing activity and it can be used to treat different types of wounds in human beings too.

REFERENCES

[1] Akhtar N, Ali M, Alam S, *Nat Prodt Res*, **2010**, 24, 962-972.
[2] Ali M A, Mozid M A, Yeasmin M S, Khan A M and Sayeed M A, *Res J Agri and Bio Sci*, **2008**, 4, 871-874.
[3] Andrews, Marti, Gallagher-Allred and Charlette, *Adv in Wound Care*, **1999**, 12, 137-138.
[4] Bergman I, Loxley R, *Anal Chem*, **1963**, 35, 1961–1965.
[5] Choi S, Kim K, Choi J, Han S, Park Y, Lee S, Kim J and Chung M, *Planta Medica*, **2002**, 68, 330-335.
[6] Dar A, Behbahaniam S, Malik A, Jahan N, *Phytomed Int J Phytother and Phytopharmacol*, **1999**, 6, 373-378.
[7] Deodhar A K, Rana R E, *J Postgrad Med*, **1997**, 43, 52–56.
[8] Diwan P V, Reddy B S, Reddy R K K. Naidu V G M, Madhusudhana K, Agwane S B, Ramakrishna S, J *Ethnopharmaco*, **2008**, 115, 249–256.
[9] Kokane D D, More R Y, Kale M B, Nehete M N, Mehendale P C and Gadgoli C H, *J Ethnopharmaco*, **2009**, 124, 311-315.
[10] Gallo M B and Sarachine M J, *Int J Biomed and Pharmaceu Sci*, **2009**, 3, 47-66.
[11] Govindrajan R, Kumar B, Vijaykumar M, Pushpangadan P, *J Ethnopharmaco*, **2007**, 114, 103–113.
[12] Hagermann A E, Tannin Chemistry in Tannin handbook, USA, **2002**, 36-42.
[13] Koti B, Ashok P, *Int J Green phar*, **2010**, 4, 90-92.
[14] Mali R G, Mahajan S G and Mehta A A, *Pharmacog mag*, **2007**, 3, 73-76.
[15] Mandelbaum S H, Di Santis E P, Mandelbaum M H S A, *Anais Brasileiros de Dermatologia*, **2003**,78, 393–410.
[16] Misra G and Mitra C R, *Phytochemistry*, **1967**, 6, 1309.
[17] Mukherjee P K, Verpoorte R, Suresh B, *J Ethnopharmaco*, **2000**, 70, 315–321.
[18] Muthusamy S K, Kirubanandan S, Sripriya, Sehgal P K, *J Surg Res*, **2008**,144, 94–101.
[19] Nayak B S, Anderson M, Pereire P, *Fitoterap*, **2007**, 78, 540–544.
[20] OECD guidelines for testing of chemicals, *Acute Dermal Toxicity* 402, **1987**, 1–7.
[21] Patra A, Jha S, Murthy P, Manik and Sharon A, *Int J Pharm Sci and Res*, **2010**, 1, 95-100.
[22] Rangama B N L D, Abayasekara C L and Panagoda G J, M.R.D.M. Senanayake, *J.Natn.Sci.Foundation Sri Lanhka*, **2009**, 37, 139-145.
[23] Ruikar A ,Torane R ,Tambe A ,Puranik V,Deshpande N, *Int J Chem Tech Res*, **2009**,2, 158-161.
[24] Schwartz S I, Schwartz's Principles of Surgery, The McGraw-Hill Publishing House, New York, **2006**, 1-5.
[25] Shah P J, Gandhi M S, Shah M B, Goswami S S , *J Ethnopharmaco*, **2003**, 89, 305-311.
[26] Yu L, Perret J, Harris M, Wilson J, Haley S, *J Agri and Food Chem* **2003**,51, 1566–1570.