WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 06, 530-540. Research Article ISSN 2277–7105

WOUND HEALING EFFECT OF METHANOLIC EXTRACT OF SYZYGIUM CUMINI (L.) LEAVES AND ITS COMBINATION WITH CLARIFIED BUTTER GHEE IN ALBINO WISTAR RATS

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Article Received on
30 Jan. 2018,
Revised on 20 Feb. 2018,
Accepted on 10 March 2018
DOI: 10.20959/wjpr20186-11356

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ABSTRACT

Syzygium cumini L., commonly known as Jamun/Jambolan, are consumed widely in India. However, little is known about wound healing properties of leaves. Present study was aimed to evaluate effect of methanolic extract of *syzygium cumini* L. (MESC) leaves and its combination with clarified butter ghee (G) using incision and excision model on albino wistar rats. Acute toxicity studies revealed the non-toxic nature of MESC and previous articles also report the cow ghee is non-toxic. Wistar albino rats (150 − 200 gm) were randomly divided into six groups (n = 6), namely Vehicle control, Framycetin skin cream (STD), MESC 200 mg/kg, MESC 400 mg/kg, MESCG 200 mg/kg (Methanolic extract of syzygium cumini L. and ghee) and MESCG 400mg/kg all doses applied topically once in a day. In incision model treatment were given for 9 days from day of wound and the tensile strength of wounds were estimated. In excision model, treatment given till 16th day of wound and percentage of wound closure and period of epithelization was calculated. Data was analyzed by unpaired t test and $p < 0.0001$ is considered as significant. Topical application of low and high dose of MESC and MESCG showed the wound healing potential which promoted wound contraction, reduced the wound closure time and increase in tensile strength. Thus, the present study support syzygium cumini L. leaves in the management of wounds and additionally ghee may decrease the form scar of wound.

KEYWORD: *Syzygium cumini*, Ghee, Wound closure, Epithelization, Tensile strength, Incision, Excision, MESC, MESCG.

INTRODUCTION

The World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare.[1] The aim of herbal treatment is usually to produce persisting improvements in wellbeing. Practitioners often talk in terms of trying to treat the "underlying cause" of disease and may prescribe herbs aimed at correcting patterns of dysfunction rather than targeting the presenting symptoms.[2] A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process that results in the restoration of anatomic continuity and function. It is a finely orchestrated and overlapping sequence of events involving – control of infection, resolution of functional connective matrix, contraction, resurfacing, differentiation and remodeling.[3] Wounds are generally classified as wounds without tissue loss (e.g. in surgery) and wounds with tissue loss, such as burn wounds. Wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments eg: venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions.[4]

Wound healing involves complex series of interactions between different cell types, cytokine mediators and the extracellular matrix. The phases of normal wound healing include haemostasis, inflammation, proliferation and remodeling.[5] Many medicinal plants have a very important role in the process of wound healing. Plants are potent healers because they promote the repair mechanisms in the natural way. Plant‐based therapy not only accelerates healing process, but also maintains the aesthetics. More than 70% of wound‐healing pharma products are plant based, 20% are mineral based and remaining contains animal products as their base material. The plant base materials are used as first aid – antiseptic coagulants and wound wash. In recent times, focus on plant research has increased all over the world and large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5‐year period.[6] Plants have been used for medicinal purposes for as long as history has been recorded. India is inhabited by wide varieties of tribal populations who dwells in forested areas and depend on surrounding resources for their livelihood. Various tribes used *Syzygium cumini* (L.) leaves for many of their ailments such as antidiabetics,[7] anticancer activity,[8]

Inflammation,[9] Antibacterial activity,[10] antifungal[11] ulcer protective and antimicrobial activity,[12] and Several studies have indicated antioxidant and free radical scavenging activity[13] of *Syzygium cumini* (L.) Leaves extract. The herbal plants of *Syzygium cumini* (L.), belong to family Myrtaceae, is commonly known as Jamun in Hindi. The present study is taken up to evaluate ayurvedic herbal plants leaves with clarified butter ghee combination to get additive effect in the process of wound healing because cow ghee shown wound healing activity.[14] Plants are more potent healers because they promote the repair mechanisms in the natural way. So the proposed study designed to evaluate effect of methanolic extract of *syzygium cumini* L. (MESC) leaves and its combination with clarified butter ghee (G) using incision and excision model on albino wistar rats.

MATERIAL AND METHOD

Materials

Collection and Authentication of plant material

The aerial parts of plant *Syzygium cumini* (L.) leaves were collected in March 2014 from Nizamabad and authenticated from Department of Botany, Osmania University, Hyderabad with voucher No.0271. Then they were shade dried and grounded coarsely and stored in air tight containers.

Extraction of plant Material

The plant leave was shade dried and coarsely powdered leaves (40gm) were packed in a soxhlet apparatus and extracted using 400 ml methanol as solvent. After extraction, the extract was obtained by distillation and dried naturally. The extract was then stored at 4°c in a refrigerator. The yield was found to be ethanolic extract 8.4% w/w.

Preliminary phytochemical study

Preliminary phytochemical screening of methanolic extract of *Syzygium cumini* (L.), leaves was done for the presence of flavonoids, steroids, alkaloids, glycosides, tannins, and phenolic compounds according to the procedures described in "Text book of Practical Pharmacognosy" by C.K. Kokate.[15]

Acute dermal toxicity study

Acute dermal toxicity studies were performed according to the OECD Guideline no. 423. Acute dermal toxicity studies of the methanolic extract of Syzygium Cumini did not exhibit any signs of toxicity up to dose 2000mg/kg body weight. Since there was no mortality of the

animals found at highest dose, 1/10th dose i.e., (200mg/kg) and 1/5th dose i.e., (400mg/kg) of the extract has been fixed as ED50 highest and lowest doses respectively for both anti-psoriatic activity and wound healing activity.

Animal selection

Thirty six male Albino Wistar rats weighing 180-300 gms for wound healing activity were obtained from National Institute of Nutrition, Hyderabad. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3° C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2014-15/MPCOL/06).

Preparation of extract solution

Solution was prepared by considering 5:1 ratio of methanolic extract of *Syzygium cumini* (L.) leaves and water and 6 drops were applied topically.

Preparation of combination of methanolic extract and ghee emulsion

4:2:1 ratio is considered to prepare an emulsion and 6 drops were applied topically on the wound. Four parts of methanolic extract of Syzygium cumini (L.) leaves mixed in 1 part of water further triturated in two parts of ghee.

Standard drug

Soframycin® Skin cream applied topically on wound containg framycetin sulphate 1% w/w as active ingredient in 30g tube manufactured by Aventis Pharma LTD.

Incision Wound Model

Male Wistar albino rats were anesthetized and one para vertebral-long incision was made through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on the depilated back of the rat. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The Rats were randomly allocated into 6 groups of six rats in each for incision wound experimental model. The animals were grouped as follows: Vehicle control (Saline solution), Positive control (framycetin sulphate, 1% w/w), Lower dose of Methanolic Extract of *S.cumini* leaves, 200mg/kg (MESC200), Higher dose of Methanolic

Extract of *S.cumini* leaves, 400mg/kg (MESC400), Lower dose Methanolic Extract of *S.cumini* leaves and ghee, 200mg/kg (MESCG200) and higher dose of Methanolic Extract of *S.cumini* leaves and ghee, 400mg/kg (MESCG400). Framycetin sulphate (1% w/w) was chosen as a standard drug for comparison because it is one of the most commonly used antibacterial drug for treating wounds. Framycetin was applied to the wound surface using a plastic spatula and the extract preparations were applied using a dropper. About 0.5 g/day framycetin and 6 drops of extract solution were applied to the wound surface of animals in their respective groups. The extract preparations were applied to the wound surface once daily for 9 consecutive days. After application of the formulations, the wound was covered with cotton gauze held in place with an adhesive tape. Animals were euthanized on the 10th day using solid carbon dioxide and the tensile strength estimations of wounds were carried out on the intact animal by local made tensiometer.[16]

Estimation of Wound Parameters

Thoroughly the sutures were removed on the 10th day and tensile strength was measured with a local made Tensiometer.

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

In the present study experiment local made Tensiometer was used, which consists of a wooden board to which four nail was fixed. To one end the nail thread tied which is fixed, were as to another end easy movement of thread was allowed with help of pulley, to the edge of thread weighing balance was attached. Two clamps were tied to the thread in each side. The rats were anesthetized individually and were placed in wooden board between nails. The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5 cm away from the wound. Analytical weights were placed on the weighing balance by increasing the weights until the healed wound opens. Thus tensile strength of wound was measured.[17]

Excision Wound Model

Animals in each group were anaesthetized by open mask method with anesthetic ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm^2 full thickness of skin from a predetermined area. The wound was left undressed to the open environment. Then the extracts and standard were administered topically for 16 days.

Contractions, which contribute for wound closure, were studied by tracing the raw wound. Wound area was measured by retracing the wound on a milli meter scale graph paper every alternate day. The degree of wound healing was calculated.[16]

Wound contraction was calculated as percent reduction in wound area using following formula.

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

Where N = Number of days 2nd, 4th, 8th, 12th, and 16th day.

Period of epithelization was also calculated and compared with that of control group.

Statistical Analysis

The data were analyzed by student unpaired t test and these were considering as significant value as compare with vehicle control and standard.

RESULT

Phytochemical investigation of methanolic extracts of the test plant showed the presence of flavonoid, steroid, glycosides, alkaloid tannins and phenolic compound. The details of qualitative chemical tests and phytoconstituents present in the extracts (Table I). The tensile strength of high (MESC400) and low dose (MESC200) of methanolic extract of *syzygium* cumini and high (MESCG400) and low dose (MESCG200) of combination of methanolic extract of *syzygium cumini* and ghee treated wound (274.6±1.78, 174.6±0.99, 695.6±3.27 and 309.8±1.19 respectively) were significantly greater ($p<0.0001$) than that of vehicle control (164±0.93). The above observation for tensile strength shows that the higher dose MESCG400 treated wound greater than that of standard drugs framycetin treated wound significantly ($P<0.0001$) (Table II). In excision wound model, period of epithelization was observed on day 0, 2,4,6,8,10,12,14 and 16^{th}. Period of epithelization on 16^{th} day was observed significantly in treated groups and standard drug treated group as compared with vehicle control. The period of epithelization was observed faster in MESC400 (0.008±0.004, $P<0.0001$) and MESCG400 (0±0, $p<0.0001$) as compare with vehicle control (Table III). The percentage wound closure was observed such as 81.4, 99.94, 96.6, 99.84, 98 and 100 on 16^{th} day in vehicle control, STD, MESC200, MESC400, MESCG200 and MESCG400 treated groups respectively. (Table IV)The complete wound closure with removal of scar on wound surface area was observed in MESCG400.

Table I: Preliminary phytochemical screening of methanolic extract of *Syzygium cumini* leaves.

Phytochemical constituents	Test	Result
Flavonoids	Shinoda Test	+++
Steroids	Salkowski's Liberman-Burchard's test	+++ +++
Glycosides	Legal test Baljet test	++ ++
Alkaloid	Dragonodroff's test Mayer's test Hager's test Wagner's test	++ ++ ++ ++
Tannin & Phenolics	Ferric chloride solution Lead acetate solution Gelatine solution Acetic acid solution	+ + + +
(+) =present, (++) = moderately present, (+++) = appreciable amount		

Table II: Tensile Strength exhibited by methanolic extract of *syzygium cumini* leaves and its combination with ghee in incision wound model on rats.

Groups	Tensile strength
Vehicle Control	164±0.93
STD (Framycetin sulphate)	428.3±2.46[a]
MESC200	174.6±0.99[b]
MESC 400	274.6±1.78[c]
MESCG200	309.8±1.19[d]
MESCG 400	695.6±3.27[e]
Values are expressed as mean ± SEM, (n=6), Data was analyzed statistically by unpaired t test. [a]p<0.0001,[b]p<0.0001,[c]p<0.0001 when compared with control group.	

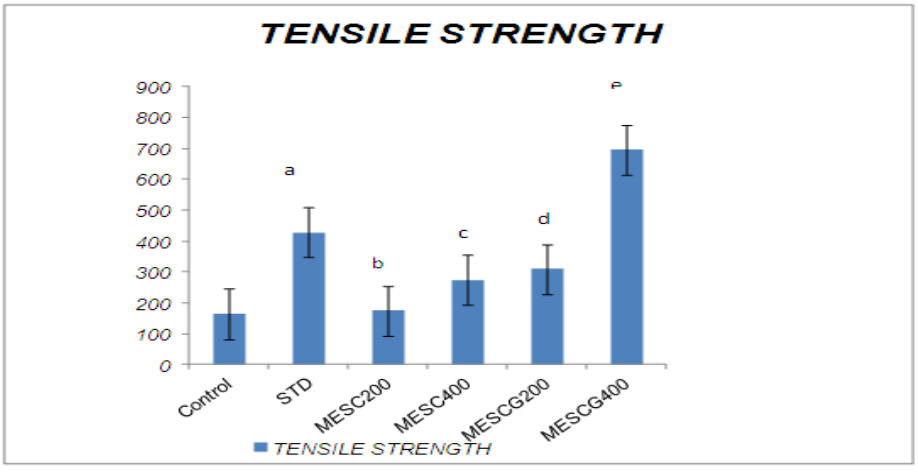

Fig 1 Tensile strength exhibited by MESC and MESCG.

Table III: Period of epithelization exhibited by methanolic extract of syzygium cumini leaves and its combination with ghee in excision wound model on rats.

Day-s	Vehicle Control	STD	MESC200	MESC400	MESCG200	MESCG400
0	5±0	5±0	5±0	5±0	5±0	5±0
2nd	4.8±0.02	4.4±0.02	4.8±0.02	4.7±0.01	4.7±0.01	4.5±0.02
4th	3.6±0.02	3.2±0.01	3.4±0.01	3.2±0.02	3.3±0.02	3.2±0.01
6th	3.4±0.02	3.0±0.03	3.0±0.03	3.0±0.02	3.2±0.05	3.2±0.01
8th	3.4±0.02	2.9±0.02	3.0±0.02	2.88±0.01	2.7±0.02	2.2±0.01
10th	*3.0±0.03*	*1.5±0.02*	*2.8±0.02*	2.0±0.02	2.0±0.03	1.02±0.01
12th	2.4±0.02	0.8±0.01	1.5±0.008	1.1±0.03	1.3±0.01	0.9±0.01
14th	1.2±0.01	0.05±0.003	0.7±0.005	0.5±0.01	0.5±0.01	0.003±0.002
16th	0.93±0.02	0.003±0.002*	0.17±0.009[f]	0.008±0.004[g]	0.1±0.02[h]	0±0[i]

Values are expressed as mean ± SEM, (n=6), Data was analyzed statistically by unpaired t test. *p<0.0001 STD compared to the control, [f]p≤0.0001 MESC200 compared to control, [g] p≤0.0001 MESC400 compared to control, [h]p≤0.0001 MESCG200 compared to control, [i]p<0.0001 MESCG400 compared to the control.

Table IV: Wound contraction area exhibited by methanolic extract of syzygium cumini and its combination with ghee in excision wound model on rats.

Post wound day		(% wound contraction)				
	Vehicle control	STD	MESC200	MESC400	MESCG200	MESCG400
0	0	0	0	0	0	0
2	4	12	4	6	6	10
4	28	36	32	36	36	36
6	32	40	40	40	36	36
8	32	42	40	42.4	46	56
10	40	70	44	60	60	79.6
12	52	84	70	78	74	82
14	76	99	86	90	90	99.94
16	**81.4**	**99.94**	**96.6**	**99.84**	**98**	**100**

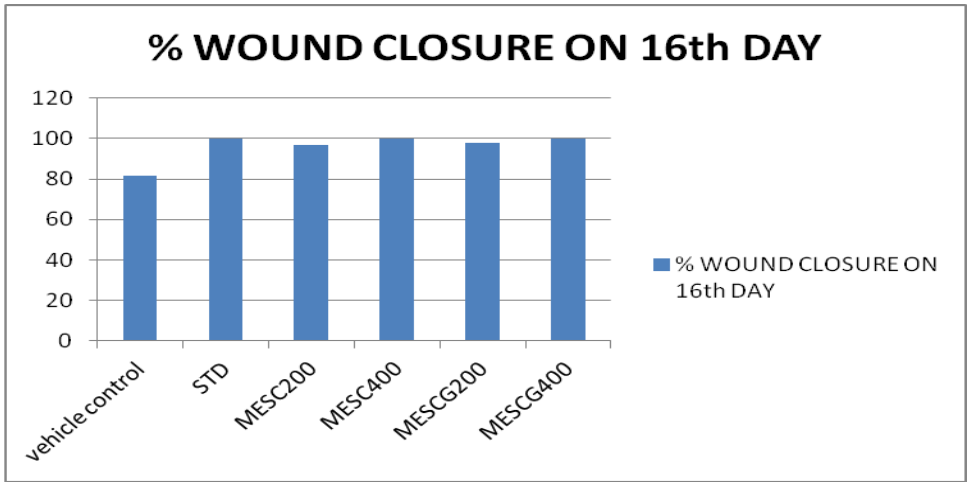

Fig 2: Percentage of wound closure on 16th day in excision wound model.

DISCUSSION

The preliminary phytochemical screening showed various bioactive ingredients such flavanoid, steroid, glycosides, alkaloid, tannins and phenolic compound. The presence of these components is an indication that this plant has some medical properties. Tannins are antibacterial compounds which damage the bacterial cell wall.[18] Phenolic compounds, alkaloids, flavonoids, tannins, saponins and glycosides are good antioxidant compounds and controls the oxidative stress related disorders.[19,20] Wound healing involves various phases. Initially involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which are later removed to form a scar.[21] Collagen matrix remodeling occurs throughout the healing process and requires abundant amounts of ascorbic acid for the post-translational hydroxylation of proline.[22] In present incision wound models study result tensile strength were observed and interpreted that low dose and high dose of MESC (200 and 400 respectively) and combination of plant extract and ghee low dose and high dose MESCG (200 and 400 respectively) showing better wound healing activity as compared to vehicle control group but all doses shown quite lesser tensile strength as compared to framycetin sulphate 1% w/w cream treated group except high dose of MESCG400. In excision wound model plant extract and its combination with Ghee (MESC and MESCG) both showed significant wound healing activity in low and high dose, but high dose of MESC400 and MESCCG400 exhibited better wound healing activity when compared to that of low dose in both parameters measures *i.e.* wound closure and period of epithelization. For the MESCG400 wound closure was 100% as well as complete period of epithelization was obtained on 16th day. Hence it may consider as high dose combination of plant extract and ghee has better wound healing activity than all other group and it expressed that it involved in collagen matrix remodelling for wound healing and also in the removal of form scar. Past study was interpreted that the wounds treated with Madhu Ghrita (MG) (combination of honey and ghee) and Framycetin Sulphate creame 1% w/w were promoting the healing marker and showing better wound healing activity. MG promotes keratinization, fibrosis, collagen formation and neovascularisation to a greater extent.[23] Ghee show better wound healing[24] as well as immunostimulant activity.[25] Cow's ghee exhibit antiulcer activity and also effective against infection in the eyes.[26] In present study combination of methanolic extract of plant and ghee may attribute in healing of wound and also involved in removal of form scar due to antioxidant and anti-inflammatory property of *syzygium cumini* L. and antimicrobial, antiulcer and immunostimulant properties of ghee.

CONCLUSION

From the study carried out showed that the methanolic extract of *syzygium cumini* L. and its combination with ghee possesses a definite wound healing activity, there by justifying its use in the indigenous system of medicine.

REFERENCE

1. Jacqueline A, Hart R, Lynn Shumake. Systems to Rate the Strength of Scientific Evidence University of Maryland Medical Center. Glenwood, 2002; 40-45.
2. Andrew Vickers, Catherine Zollman. BMJ, 1999; 319: 1050-1053.
3. Martin P. wound healing aiming for perfect skin regeneration. Science, 1997; 276: 75-81.
4. Nwomeh BC, Yager DR, Cohen IK. Physiology of the chronic wound. Clin Plast Surg, 1998; 25: 341–356.
5. Douglas M and Alan LM. Support for wound healing. Alter Med Rev, 2003; 8: 359-60**.**
6. Kumarasamyraja D, Jeganathan NS, Manavalan R. A review on medicinal plants with potential wound healing activity. Int J Pharma Sci, 2012; 2: 105-11.
7. Alam et.al, Evaluation of antidiabetic phytochemicals in *Syzygium cumini* (L.) Skeels (Family: Myrtaceae); Journal of applied pharmaceutical science, 2012: 2(10): 094-098.
8. Parmar J, Sharma P, Varma P, Goyal PK. et.al., Chemopreventive Action of *Syzygium cumini* on DMBA-induced Skin Papillomagenesis in mice. Asian pacific journal of cancer prevention, 2010; 11: 261-65.
9. Rachel R P Machado et al. the effect of essential oil of syzygium cumini on the development of granulomatous inflammation in mice. Brazilian journal of pharmacognosy, 2013; 23(3): 488-496.
10. Joji R, Beena J et al., evaluation of antibacterial and DPPH radical scavenging activities of the leaf extracts and leaf essential oil of syzygium cumini linn. from south india. International journal of pharmacy and pharmaceutical sciences, 2013; 5(3): 2013.
11. Zia U. K, Harbans S. Randhawa, Tusharantak K, et al., Antifungal susceptibility of Cryptococcus neoformans and Cryptococcus gattii isolates from decayed wood of trunk hollows of Ficus religiosa and Syzygium cumini trees in north-western India. Journal of Antimicrobial Chemotherapy, 2007; 60: 312–316.
12. Bhargava P and Jain U K, Bhargava S et. al., Evaluation of ulcer-protective and antimicrobial activity of *Syzygium cumini* (linn.) skeels leaves. Pharmacology_online, 2009; 3: 266-274.

13. Zhi Ping R, Liang Liang Z and Yi Ming L, Evaluation of the Antioxidant Activity of *Syzygium cumini* Leaves. Molecules, 2008; 13: 2545-2556.
14. Biyani DM, Verma1 P R P., Dorle A K,. Boxey V. A Case Report on Wound Healing Activity of Cow Ghee, International Journal of Ayurvedic Medicine, 2011; 2(3): 115-118.
15. Kokate CK. Practical Pharmacognosy, Preliminary Phytochemical Screening, first edition. New Delhi: Vallabh Prakashan, 1986: 111.
16. Shilpa K, Rajendra Y, Sanjeeva kumar A, Vinay kumar D, et al. Evaluation of wound healing potential in crinium defixum ker gawl bulbs k. asian j pharm clin res, 2013; 6(1): 61-63.
17. Santram L, Gautam PV, Relevance And Perspectives Of Experimental Wound Models In Wound Healing Research. Asian J Pharm Clin Res, 2017; 10(7): 57-62.
18. Mainasara MM, Aliero, BL, Aliero AA and Yakubu M. Phytochemical and Antibacterial Properties of Root and Leaf Extracts of *Calotropis procera*. Nigerian J. Basic Appl. Sci, 2012; 20(1): 1-6.
19. Amal A. Mohamed, S I. Ali, and Farouk K E. Antioxidant and Antibacterial Activities of Crude Extracts and Essential Oils of Syzygium cumini Leaves. PLoS One, 2013; 8(4): e60269.
20. Biapa, PC, Agbor, GA, Oben, JE and Ngogang, JY. Phytochemical studies and antioxidant properties of four medicinal plants used in Cameroon. Afr. J. Trad. Complement. Altern. Med, 2007; 4(4): 495-500.
21. Chithra P, Sajithalal BG, Gowri C. Influence of Aloe vera on collagen turnover in healing of dermal wounds in rats. Ind J Exp Biol, 1988; 36: 896-901.
22. Cetin E O, Yesil-Celiktas O, Cavusoglu T, Demirel-Sezer E, et al,. Incision wound healing activity of pine bark extract containing topical formulations: A study with histopathological and biochemical analyses in albino rats. Pharmazie, 2012; 67: 1–6.
23. Charde MS, Fulzele SV, Satturwar PM, et. al. Wound healing and anti-inflammatory potential of madhu ghrita. Indian J Pharm Sci, January, 2006; 68(1): 26-31.
24. Prasad V, Dorle AK. Evaluation of ghee based formulation for wound healing activity. J Ethnopharmacology, 2006; 107(1): 38-47.
25. Fulzele SV, Bhurchandi PM, Kanoje VM, et. al. Immunostimulant activity of Ashthamangal Ghrita in Rats. Ind J Pharmacol, 2002; 34: 194-197.
26. Biyani DM, Verma PRP., Dorle AK, et. al. A Case Report on Wound Healing Activity of Cow Ghee, International Journal of Ayurvedic Medicine, 2011; 2(3): 115-118.