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EVALUATION OF THE WOUND HEALING ACTIVITY OF THREE SELECTED MEDICINAL PLANTS OF SOUTH INDIA

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ABSTRACT

Wound healing is a complex biological process involving inflammation, cell proliferation, collagen deposition, and tissue remodelling. Medicinal plants used in traditional systems are increasingly explored as alternative therapeutic agents due to their bioactive constituents and minimal side effects. The present study aimed to evaluate the wound healing potential of three selected South Indian medicinal plants—*Mimosa pudica*, *Artabotrys hexapetalus*, and *Adhatoda vasica*—using an excision wound model in albino rats. Methanolic, chloroform, and diethyl ether extracts of the plants were formulated into 10% (w/w) ointments and compared with a standard nitrofurazone ointment (0.2% w/w) and a control base. Wound contraction and epithelialization time were assessed over a 16-day period. All plant extracts demonstrated significant wound healing activity compared to the control. Among the extracts, methanolic formulations showed superior efficacy, with *M. pudica* exhibiting the highest wound contraction (93.87%), followed by *A. vasica* (87.46%) and *A. hexapetalus* (78.61%). Chloroform and diethyl ether extracts showed moderate to lower activity. The enhanced healing effect of methanolic extracts may be attributed to the presence of bioactive phytoconstituents such as flavonoids, alkaloids, tannins, and phenolic compounds that promote collagen synthesis and tissue regeneration. The findings support the traditional use of these plants and highlight *M. pudica* methanolic extract as a promising candidate for the development of herbal wound-healing formulations.

Keywords: Wound healing, medicinal plants, *Mimosa pudica*, *Artabotrys hexapetalus*, *Adhatoda vasica*, excision wound model

INTRODUCTION

In 1863, Virchow (Balkwill et al., 2001) proposed that some irritants had the potential to cause cancer by causing inflammation. This was later discovered to be related to irritants and tissue damage, both of which are necessary for wound healing and result in increased cell proliferation. These inflammatory elements gradually fade away as the wound heals. However, because of the persistent inflammation and the availability of inflammatory factors as well as other agents, including DNA damaging agents, certain cells may incur mutations and continue to proliferate in the nutrient-rich microenvironment, leading to cancer (Coussens and Werb, 2002). A number of causes, including autoimmune disorders (colon cancer–inflammatory bowel disease), microbiological factors (gastric cancer–Helicobacter pylori infections), and miscellaneous factors (prostate cancer–prostitis), cause cancer to be triggered by inflammation.

Many variables, including viral infections, cause sub-threshold neoplastic conditions, according to Peyton Reus (Rous, 1910). This is known as the "initiation" stage of cancer. Secondary signals, such as irritants and compounds such as phorbol esters and chemicals generated at the site of wound healing, organ

resection, and so on, are constantly present during cancer. This is known as the "promotion" stage. This is the stage where the cells with the mutations continue to proliferate in the presence of numerous inflammatory stimuli, eventually resulting in a tumour (Cossens and Werb, 2002).

In the inflammatory microenvironment, both in the supporting stroma and the tumour, host leucocytes such as macrophages, dendritic cells, and lymphocytes are present (Lu et al., 2006). Mast cells in the tissues have also been found to play a key function in inflammation. All of these elements work together to create a provisional extracellular matrix in which endothelial and fibroblast cells may develop, as well as an environment in which "promoted" cells can thrive. These circumstances are also present during the healing of wounded tissues' wounds. Platelet aggregation causes the release of thrombin, which starts the blood clotting process and prevents the loss of nutrients. Apart from that, platelet aggregation triggers inflammatory processes by secreting a variety of proteins and -granules to the damaged area, causing inflammation. During chronic inflammation, the process continues, potentially leading in mutations and a favourable milieu for cancer cells to thrive, resulting in tumour formation (Cossens and werb, 2002). Tumors, according to Dovorak (1986), are wounds that do not heal.

Inflammatory diseases come in a variety of forms. Some of them aren't dangerous to your health. However, some inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, have the strongest link to colon tumour formation. Aside from these, schistosomiasis plays a significant role in colon carcinoma, whereas persistent infection with *H. pyroli* is the principal cause of stomach cancer. The Gram-negative bacteria have been identified as the cause of stomach cancer, with DNA damage resulting from persistent inflammation thought to be the mechanism. Hepatocarcinoma development is also influenced by hepatitis C infection in the liver. The effect of several plants extracts on probable inflammatory damage sites such as the liver (hepato protection), stomach (ulcer), and external wounds (wound healing) in terms of immune regulation was investigated in this thesis. Aside from that, the potential anti-oxidative capabilities of different extracts have been investigated.

Many chemotherapy drugs work by having anti-proliferative and cytotoxic properties. However, the same medicines were capable of immunosuppression since the treatment inhibits the rapid expansion of immune cells. Through the manipulation of the immune system, several medicines with immunomodulation properties have improved cancer treatment (Ehrke and Jane, 2003). The immunomodulatory medication cyclophosphamide is an excellent example. At greater doses, the medication has varied effects, but whether provided alone or in combination with other drugs, it exhibits anti-cancer and immunomodulatory capabilities and has cured cancer in mice models. Aside from cyclophosphamide, various additional drugs have been proven to influence the immune system, including nitrosoreus compounds like adriamycin, arabinosylcitrosine, and others (Ehrke et al., 1996).

MATERIAL AND METHODS

Collection of Plant Materials: During the early winter season, the plant materials *M. pudica, A. hexapetalus*, and *A. vasica* were obtained from the foothills of the Western Ghats in and around Courtallum and Thaniparari Hills, Tamil Nadu, India.

Experimental Animals: Male *albino* rats weighing 150-200 g were fed a regular food (Pranav Agro, India) and were kept in the institutional animal house facility under conventional laboratory conditions.

Method of Preparation: The materials were combined and gently cooked, stirring constantly, until a homogeneous mixture was achieved. The contents of the previous section were cooled to room temperature. The ointment with a 10% concentration was made. In the case of plant extracts, 1 gm of appropriate extract was combined with 10 gms of ointment base (10%) and well mixed until a homogeneous ointment was created.

Types of ointment prepared: Eleven types of ointments were prepared as indicated below:
1. Simple ointment base - Control
2. 0.2% w/w of Nitrofurazone ointment -Standard

3. Base + Methanolic Extract of *M. pudica* (10% w/w)
4. Base + Chloroform Extract of *M. pudica* (10% w/w)
5. Base + Diethyl Ether Extract of *M. pudica* (10% w/w)
6. Base + Methanolic Extract of *A. hexapetalus* (10% w/w)
7. Base + Chloroform Extract of *A. hexapetalus* (10% w/w)
8. Base + Diethyl Ether Extract of *A. hexapetalus* (10% w/w)
9. Base + Methanolic Extract of *A. vasica* (10% w/w)
10. Base + Chloroform Extract of *A. vasica* (10% w/w)
11. Base + Diethyl Ether Extract of *A. vasica* (10% w/w)

Excision Wound Model: For each of these studies, male Albino rats weighing 150-200 grammes were chosen and divided into 11 groups of five individuals. The animals were kept in an experimental room that was kept in accordance with IAEC guidelines. The experimental animals were anaesthetized with lignocaine injections at a concentration of 2% across a localised area. The rats were depilated and an excision wound was created by cutting away 500 mm square thickness of skin from the predetermine area, the wound was left open then the drugs, reference standard (0.2 % w/w Nitrofurazone ointment), control (simple ointment base B.P) and methanol, chloroform and diethyl ether extracts of 3 plants ointment (*M. pudica*, *A. hexapetalus* and *A. vasica)* were applied until the wound was healed. This model was used to monitor the wound contraction and wound closer time. The wound contraction was calculated as percentage reduction in wound area. The progressive change in wound area is monitored by calculating the decreasing area (Muppayavarmath and Patil, 1999).

RWH = Size of Wound in surface area (mm^2) at Day 16 / Size of Wound in surface area (mm^2) at Day 1 X 100
% Reduction in Healing = 100 – RWH

Fractionation of *Mimosa pudica* Extract: Liquid–liquid extraction, also known as solvent extraction or partitioning, is a technique for separating chemicals based on their relative solubility in two immiscible liquids, often water and an organic solvent. It is the process of extracting a material from one liquid phase into another. The following immiscible solvent combinations were used to fractionate the methanolic extract of *M pudica* in a 1:1 ratio (purified water: hexane, purified water: ethyl acetate, purified water: chloroform and purified water: n-butanol).

Protocol: In a first separating funnel, two grammes of methanolic extract of *M. pudica* were dissolved in 10 litres of solvent filtered water and an equivalent volume of hexane was added. This was shook for 10 minutes before being allowed to settle or centrifuged for 15 minutes at a low speed. The bottom and top phases were separated into separate containers. Separately evaporate the two liquid stages. Use alternative solvent mixes, such as pure water: ethyl acetate, purified water: chloroform, and purified water: N-butanol, to repeat the operation. The wound healing activity was assessed using a combination of aqueous extracts and organic extracts (McCraken and Chaikin, 1974).

RESULTS

Wound healing activity was seen in all three plant extracts. The diethyl extract had a reduced wound healing capacity, but the methanolic extracts had a higher activity. *M. pudica* methanolic extract had a greater activity of 93.87 percent, whereas *A. hexapetalus* and *A. vasica* methanolic extracts had 78.61 percent and 87.46 percent, respectively.

***M. pudica* (MP)**

The wound healing activity was studied by using five groups; Group I negative control simple ointment, In Group II positive control Nitrofurazone ointment (0.2% w/w), Group III MPME, Group IV MPCE and Group V MPDEE. The size of the wound in surface area, On the Day 1 (50.24) (50.36) (51.26) (50.54) (50.42). On the Day 4 (48.24) (28.26) (38.46) (38.36) (48.46). On the Day 8 (44.20) (12.56) (28.26) (30.26) (40.32). On the Day 12 (40.46) (3.14) (12.56) (20.54) (36.16). On the Day 16 (35.46) (0.758) (3.14) (18.76) (20.45). The mean percentage closure of excision wound model on Day 16 (40.45) (98.44) (93.87) (80.72) (70.76) (Table 1; Figure 1 and 4).

***A. hexapetalus* (AH)**

Five groups were used to study wound healing activity: Group I was a negative control base ointment, Group II was a positive control Nitrofurazone ointment (0.2 percent w/w), Group III was AHME, Group IV was AHCE, and Group V was AHDEE. The surface area of the wound was measured to assess its size. On Day 1, the wound measured 48 to 50.24 square millimetres. The size of the wound in animals treated with A. vasica extracts was reduced to 6.42 sq.mm, 9.80 sq.mm, and 15.60 sq.mm after 16 days for methanol, chloroform, and diethyl ether extracts, respectively (Table 1; Figure 2 and 4).

The excision wound began to contract on Day 4 and continued to do so until Day 16. After 16 days, the % wound contraction in the nitrofurazone, methanol, chloroform, and diethyl ether extract treated groups was 98.44, 78.61, 70.82, and 65.46, respectively. In compared to the control group, all treatment groups saw significant wound contraction on the 16th day (p0.001 for standard and methanolic extracts; p0.01 for chloroform and diethyl ether extracts). In both the medication and control groups, the time to complete epithelization was dramatically reduced.

The epithelization of a wound in a rat treated with extracts occurred much sooner than in a control animal. It is likewise equivalent to the commercially available preparation. It's possible that the Artabotrys hexapetalus leaf extracts aided wound healing. The excision wound model revealed that methanolic leaf extract had good wound healing properties when compared to a conventional medication.

***A. vasica* (AV)**

The wound healing activity was studied by using five groups; Group I negative control simple ointment, In Group II positive control Nitrofurazone ointment (0.2% w/w), Group III AVME, Group IV AVCE and Group V AVDEE. The size of the wound in surface area, On the Day 1 (50.24) (50.36) (51.16) (50.62) (49.84). On the Day 4 (48.24) (28.26) (38.14) (36.90) (37.10). On the Day 8 (44.20) (12.56) (27.84) (27.52) (29.34). On the Day 12 (40.46) (3.14) (16.12) (18.42) (21.64). On the Day 16 (35.46) (0.758) (6.42) (9.80) (15.60). The mean percentage closure of excision wound model on Day 16 (40.45) (98.44) (87.46) (80.65) (68.70) (Table 1; Figure 3 and 4).

Excision wound contraction was accelerated from day 1 to day 16. On the 16th day, wound contraction for the nitrofurazone, methanolic, chloroform, and diethyl ether extract treated groups was 98.44, 87.46, 80.65, and 68.70 percent, respectively, in excision wounds. In compared to the control group, all treatment groups showed significant wound contraction on the 16th day (p0.001 for standard and methanolic extracts; p0.01 for chloroform and diethyl ether extract). In both the medication and control groups, the time to complete epithelization was dramatically reduced.

The epithelization of a wound in a rat treated with extracts occurred much sooner than in a control animal. It is likewise equivalent to the commercially available preparation. It's possible that the *Adhatoda vasica* leaf extracts aided wound healing. The excision wound model revealed that methanolic leaf extract had good wound healing properties when compared to a conventional medication.

Table 1: Effect of methanolic, chloroform and diethyl ether extract ointments of MP, AH and AV on excision wound model

Group	Avg. wt of animal	Drug /extract	Size of wound surface area (mm ^{2})						% wound healing
			Day 0	Day 1	Day 4	Day 8	Day 12	Day 16	
I	150-200 gm	Control	50.24	50.24	48.24	44.20	40.46	35.46	40.45
II		Nitrofurazone ointment (0.2% w/w)	50.36	50.36	28.26	12.56	3.14	0.758	98.44
III		MPME (10%w/w)	51.26	51.26	38.46	28.26	12.56	3.14	93.87 **
IV		MPCE(10%w/w)	50.54	50.54	28.36	30.26	20.54	18.76	80.72 **
V		MPDEEE (10%w/w)	50.42	50.42	48.46	40.32	36.16	20.45	70.76**
VI		AHME (10%w/w)	48.60	48.60	36.20	27.12	18.14	10.40	78.61**
VII		AHCE (10%w/w)	49.20	49.20	35.22	28.84	21.20	14.36	70.82**
VIII		AHDEEE (10%w/w)	50.42	50.42	38.96	32.14	24.20	17.42	65.46*
IX		AVME (10%w/w)	51.16	51.16	38.14	27.84	16.12	6.42	87.46**
X		AVCE (10%w/w)	50.62	50.62	36.90	27.52	18.42	9.80	80.65**
XI		AVDEEE	49.84	49.84	37.10	29.34	21.64	15.60	68.70*

Values are mean ± SEM of 5 animals in each group. Numbers in Parenthesis indicate percentage of wound contraction. * P<0.01, **P<0.001Vs respective control by students t- test.

a)

Control 0 day

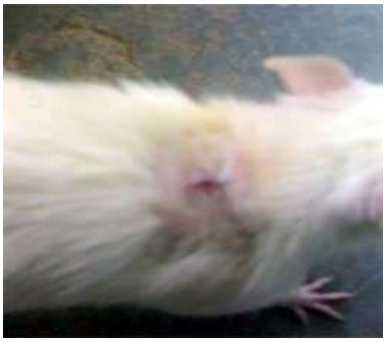
b)

Standard 0 day

c)

Control 16 day

d)



Standard 16 day

e) **f)** **g)**

Mimosa pudica (Diethyl ether Extract10%W/W)

Mim*osa pudica* (Chloroform Extract10 %W/W)

Mimosa pudica (Methanol Extract 10%W/W)

Figure 1: Effect of methanolic, chloroform and diethyl ether extract ointments of *M.pudica* on excision wound model.

a) **b)** **c)**

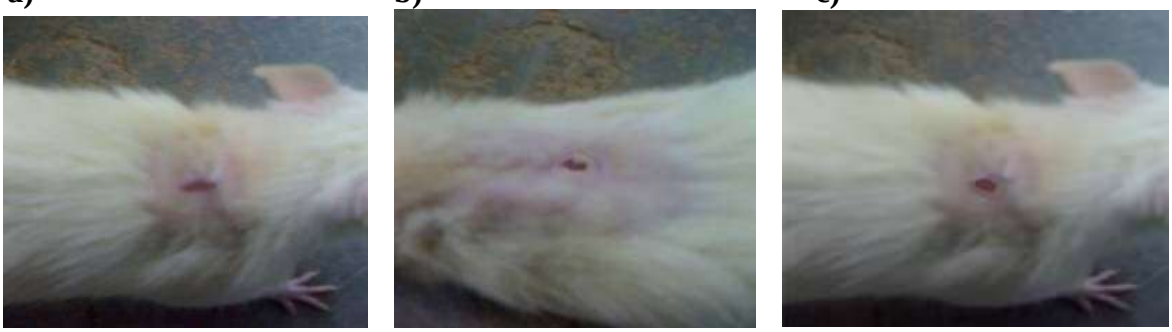
Artabotrys hexapetalus (Diethyl ether Extract10%W/W)

Artabotrys hexapetalus (Chloroform Extract10 %W/W)

Artabotrys hexapetalus (Methanol Extract 10%W/W)

Figure 2: Effect of methanolic, chloroform and diethyl ether extract ointments of *Artabotrys hexapetalus* on excision wound model.

a) **b)** **c)**

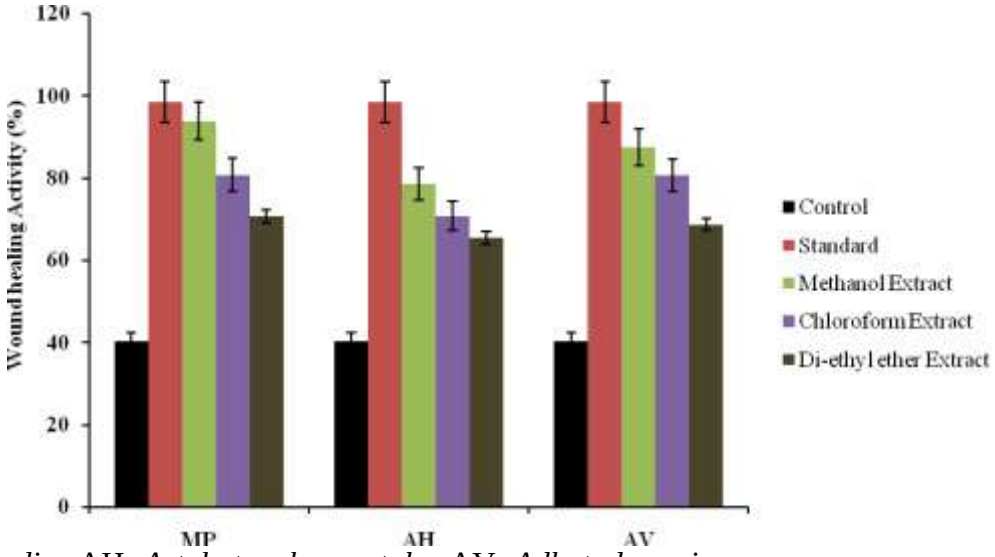


Adhatoda vasica (Diethyl ether Extract10%W/W)

Adhatoda vasica (Chloroform Extract10 %W/W)

Adhatoda vasica (Methanol Extract 10%W/W)

Figure 3: Effect of methanolic, chloroform and diethyl ether extract ointments of *Adhatoda vasica* on excision wound model.



MP- *Mimosa pudica* AH- *Artabotrys hexapetalus* AV- *Adhatoda vasica*

Figure 4: Effect of methanolic, chloroform and diethyl ether extract ointments of *MP, AH and AV* on excision wound model.

DISSCUSSION

Wound healing is a multi-stage process that includes contraction, epithelization, granulation, and collagenation, among other things. It usually begins with an inflammatory phase, which is followed by fibroblast proliferation, collagen fibre production, and shrinkage, all of which occur simultaneously but independently of one another. Several plants have been identified as having wound-healing properties. The astringent and antibacterial properties of flavonoids, glycosides, and tannins found in plant extracts are known to aid wound healing (Nayak et al., 2007). Flavonoids are also known to minimise lipid peroxidation via increasing vascularity as well as avoiding or reducing the beginning of cell necrosis. Lipid peroxidation plays a role in a variety of traumas, including burns, infected wounds, and skin ulcers. As a result, all medications that limit lipid peroxidation are thought to improve the strength of collagen fibres by reducing cell damage, boosting tissue circulation, and stimulating DNA synthesis.

Alkaloids, glycosides, flavonoids, tannins, and phenolic substances were found in preliminary phytochemical examination of the leaves of the three plant extracts; the presence of these components may contribute to wound healing activity. When compared to the other extracts, the results of this study show that the methanolic, chloroform, and diethyl ether extract ointment (10 percent w/w) of M. Pudica has substantial wound healing activity. When compared to the standard medicine, methanolic extract ointments (10 percent w/w) of the other two plants likewise had a substantial impact.

Based on the findings of this study, it is feasible to infer that the M. pudica methanolic, chloroform, and diethyl ether extract ointment (10 percent w/w) has strong wound healing activity. In the excision wound model, the methanolic extract of Mimosa pudica ointment (10% w/w) exhibited a substantial impact when compared to the reference medicine (0.2 percent w/w of Nitrofurazone ointment) and the other two extracts.

Some of the essential criteria for wound healing that would reestablish anatomical continuity and function in the damaged region include minimising tissue damage, appropriate nourishment, giving required oxygen in the wounded tissue, and maintaining a moist environment at the wound site. When comparing the wound contraction in the first four days to the control group, it is discovered that there is no significant difference. The findings of the eighth day show that the group treated with conventional medicine (nitrofurazone) and methanolic extract of Mimosa pudica ointment has a substantial rise in percentage wound contraction, indicating that the extract has the potential to drive cellular proliferation. Hydroxyproline is an amino acid that is essential for the production of protein collagen and is a key component of the collagen protein.

Collagen synthesis has been measured using hydroxyproline content as an indication.

M. pudica is a good source of steroidal and triterpenoidal saponin chemically. Constituents such as -sitosterol and triterpenoides such as Lupeol appear to play a significant role in pharmacological actions. Granulation tissue proliferation, mostly created by fibroblasts, and the angiogenesis process characterise the proliferative phase. Angiogenesis is required for the delivery of oxygen and metabolites to tissues during the proliferative phase. -Sitosterol has been shown to have a therapeutic angiogenic impact on injured blood arteries (Choi et al., 2002). - Sitosterol also has anti-inflammatory, anti-pyretic, anti-arthritic, and anti-ulcer properties in M. pudica (Patra et al., 2010). Lupeol has anti-protozoal, anti-inflammatory, and anti-microbial properties, all of which help the wound healing process. Lupeol is also utilised as an anti-inflammatory and chemopreventive agent (Gallo and Sarachine, 2009).

The findings show that the plant extracts considerably increased collagen production when compared to the control group. The use of a single model is insufficient, and there is no reference standard that can depict the numerous components of wound healing as medications that impact one phase but not necessarily the next (Sharma and Sikarwar, 2008).

CONCLUSION

The wound healing activity of the methanolic extract of *M. pudica* was greater than the other extracts. The activity of diverse extracts ranged from 65.46 percent to 93.87 percent, indicating that all three plant extracts had considerable wound healing activity. The wound healing activity of the diethyl ether extract was found to be lower, but the activity of the methanolic extracts was found to be greater. M. pudica methanolic extract had a greater activity of 93.87 percent, whereas A. hexapetalus and A. vasica methanolic extracts had 78.61 percent and 87.46 percent, respectively.

REFERENCES

1. Balkwill F and Mantovani A, (2001), “Inflammation and cancer: back to Virchow?” The Lancet, Vol. 357 (9255), pp. 539-545.
2. Cossens L.M and Werb Z, (2002), “Inflammation and cancer” Nature, Vol. 420 (6917), pp. 860-867.
3. Rous P, (1910), “A transmissible avian neoplasm.(Sarcoma of the common fowl)” The journal of experimental medicine,Vol. 12 (5), pp. 696-705.
4. LuH, OuyangW and Huang C, (2006), “Inflammation, a key event in cancer development”Molecular Cancer Research, Vol. 4 (4), pp. 221-233.
5. DvorakH.F, (1986), “Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing”The New England journal of medicine, Vol. 315 (26), pp. 1650-1659.
6. EhrkeM and Jane, (2003), “Immunomodulation in cancer therapeutics” International immunopharmacology, Vol. 3 (8), pp. 1105-1119.
7. Ehrke M.J, Verstovsek S, Zaleskis G, Ho R.L, Uihazy P, Maccubbin D.L and Mihich E, (1996),“Specific anti-EL4-lymphoma immunity in mice cured 2 years earlier with doxorubicin and interleukin-2” Cancer Immunol Immunother,Vol. 42(4), pp. 221-230.
8. Muppayavarmath S.S and Patil P.A, (1999), “The influence of tricyclic antidepressants on resutured incision and deep space wound healing” Indian J. pharmacology, Vol. 31, pp. 290-293.
9. McCraken J.R and Chaikin M, (1974), “Polarity fractionation of solvent extracted Wool Grease” Journal of the Textile Institute, Vol. 65(5), pp. 261.
10. Nayak B.S, Sandiford S and Maxwell A, (2007), “Evaluation of the wound-healing activity of ethanolic extract of Morinda citrifolia L” Evid Based Complement Alternat Med, Vol. 6 (3), pp. 351–356.
11. Choi S, Kim K.W, Choi J.S, Han S.T, Part YI, Lee S.K, Kim J.S and Chung M.H, (2002), “Angiogenic activity of β-sitosterol in the ischaemia/reperfusion-damaged brain of Mongolian Gerbil” Planta Med, Vol. 68 (4), pp. 330–335.
12. Gallo M.B and Sarachine M.J, (2009), “Biological activities of lupeol” Int J Biomed and Pharmaceu Sci, Vol. 3, pp. 46-66.
13. Sharma S and Sikarwar M.S, (2008), “Wound healing activity of ethanolic extract of leaves of Eclipta alba” Pharmacognosy Magazine, Vol. 4 (13), pp. 108-111.