Original Article

Evaluation & Herbal Formulation Of Indian Medicinal Plant On Wound Healing Activity On Experimental Animals

Harshita Srivastava[1*], Priya Mishra[2], Anupama Rao[3], Ratnanjali Pandey[4], Mukesh Kumar Shukla[5]

[1] Department of Pharmaceutics, GCRG College of Pharmacy, Parvatpur, Chandrika devi road, Bakshi ka talab, Lucknow, U.P., India
[2]Department of Pharmaceutical Chemistry, J S Singh Institute of Pharmacy, Sitapur, India
[3]Department of Pharmacognosy, R G S College of Pharmacy, Itaunja, Lucknow, U.P., India
[4]Department of Pharmacology, Hygia Institute of Pharmaceutical Education and Research, Faizullaganj, Prabandh Nagar, Ghaila Road, Lucknow, U.P., India - 226020
[5]Department of Pharmaceutics, Hygia Institute of Pharmacy, Faizullaganj, Prabandh Nagar, Ghaila Road, Lucknow, U.P., India - 226020
Email: [1]sharshita2992@gmail.com

***Corresponding Author:** Harshita Srivastava
Department of Pharmaceutics, GCRG College of Pharmacy, Parvatpur, Chandrika devi road, Bakshi ka talab, Lucknow, U.P., India
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Abstract

Acacia nilotica is an important plant commonly known as Babool and used to treat many ailments by traditional healers and local people. A. nilotica is regarded seeing that one of the most dire weeds as of its invasiveness, likely used for spread, economic with environmental impacts, thus can be easily grown and spread on large massive area for its survival recent studies and experiment shows that A.nilotica has many effective and efficient phytochemical activities in curing human disease.

This study was designed to evaluate wound healing potential of A.nilotica bark through excision wound model. A remarkable wound healing activity was observed with 20% w/w ointment of A.nilotica bark extract. The percentage of wound short form was a lot enhanced. The study provides sufficient evidence that Acacia nilotica bark might be indeed potential source to treat many diseases.

Index Terms— Acacia nilotica bark extract, excision wound model, ointment, wound healing.

INTRODUCTION

Herbal medicines are also called as botanical medicine or phytomedicine which refers to use plant's seeds, roots, leaves, berries, bark, or flowers for medicinal purpose [1]. The World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care [2]. Herbal medicine is used to treat many conditions of diseases, such as asthma, eczema, rheumatoid arthritis, premenstrual syndrome, menopausal symptoms, migraine, chronic fatigue, irritable bowel syndrome, and cancer, among others.[3] Herbal medicines can be brought without prescription and they are available in all most all health stores. Some may be grown at home. The natural detoxification process of the body is effectively enhanced by herbal medicines. They can be used to rinse out the colon get better digestion and food absorption. Herbal medicines are also good in boosting the immune system.

Wound may be defined as a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection. [4] Wound is produced due to any accident or cut with sharp edged things. Wounds are those physical injuries that result in an opening of the skin that causes disturbance in the normal skin function and skin anatomy. Pain and inflammation on the injury site is always produced with inflammatory peacekeepers of unhealed injury. Wound infections are most common in developing countries because of poor hygienic conditions. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, Corynebacterium spp., and *Pseudomonas aeruginosa* are some important organisms causing wound infection [5,6].

MATERIALS AND METHODOLOGY:

COLLECTION OF PLANT MATERIAL

The fresh bark of the *A. nilotica* was collected in the month of May 2015 from the National Botanical Research Institute Lucknow and plants were authenticated with existed live specimen present in our Institutional Garden and herbarium prepared and deposited in the laboratory for future reference.

PREPARATION OF EXTRACT

After collection and authentication, shade-dried bark (500g) of *A. nilotica*was powdered and extracted with of 50% ethanol at 50^0C on a water bath using Soxhlet extractor for 24 h. [41-43] Then extract was filtered and concentrated under reduced pressure in a rota vapor (Buchi R-200 USA) at 45^0C and then freeze-dried in lyophilizer (Labconco, USA) to obtain solid residue (ASE, yield 20.0% w/w).

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The 50% Ethanolic extract of *A. nilotica*(bark) was screened for the presence of various phytochemical constituents such as alkaloids (wager's reagent), flavonoids (shinoda test), Glycoside (Anthroquinone test), saponins (hemolysis test), tannins (ferric chloride test) and steroids (acetic anhydride test) as described by Trease and Evans and Harborne[45] according to a previously described method for extraction.

FORMULATION OF OINTMENT BASE

PROCEDURE:

Bees wax was taken in a container and heating was continued till temperature reached around 70-75ºC. At this temperature bees wax was completely melted and poured in mortar and pestle. A. nilotica bark extract was then dissolved in it and liquid paraffin was further with continuous stirring and allowed to cool. The composition of ointment formulation is given in table 1.

Table 1. The composition of ointment formulation

Ingredients	**Quantity for 10% ointment**	**Quantity for 20% ointment**
Bees wax	7.5 gms	7.5 gms
Liquid paraffin	12.5ml	11.5ml

Table 2. Optimization of *A.nilotica* bark extracts ointment

Ingredients	**Quantity for 10% ointment**	**Quantity for 20% ointment**
Bees wax	7.5 gms	7.5 gms
Liquid paraffin	12.5ml	11.5ml
Acacia nilotica bark extract	1 gm	2gms

IN VIVO STUDIE

EXPERIMENTAL ANIMALS

Albino wistar rats (150-200g) of either sex were taken from the animal house of the National Laboratory Animal Centre, Lucknow, India. They were kept under controlled conditions of temperature 27 ± 2^0C and relative humidity 44-56%, light/dark cycles of 12 hours respectively for one week before and during the experiments. Animals were provided with a standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum.* All experiments were performed in the morning accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals approved by the Institutional Committee for Ethical use of Animals and Review Board (106/IAEC/RB/7-11).

EXCISION WOUND MODEL

The particular skin area will be shaved 1 day prior to the experiment**.** The rats will be anesthetized by administrating ketamine (0.5 ml /kg, b. w. I.P.). An impression will be made on the dorsal thoracic region, 1 cm away from vertebral column and 5 cm away from ear, using a round seal of 2.5 cm diameter on anesthetized rat. The skin of impressed area will be excised with the help of forceps and sharp scissor in circular area (Approx.500 mm2) and upto depth of 2mm, 30 minute later the administration of ketamine injection. Hemostasis will be achieved by blotting the wound with cotton swab soaked in normal saline. The wounds will be left undressed. The wounding day is considered as day 0. 24 albino rats of either sex were used for excision wound model and the ointment is applied topically and animal were divided into following groups:

Group- I: 10% w/w ointment was applied once daily.

Group- II: 20% w/w ointment was applied once daily.

Group- III: Betadine 10% w/w ointment was applied once daily.

Group- IV: only ointment base was applied once daily.

Six animals were taken in each group; all the above mentioned treatments were started from the day of operation and continued till the 15th days of healing. On 0 day, 3rd day, 6th day, 9th day, 12th day and 15th day the wound area of each rat was traced on a graph paper and measured with the help of scale.

EVALUATION

IN VITRO EVALUATION

SPREADABILITY OF OINTMENT

Spreadability was determined by modified wooden block and glass slide apparatus. The apparatus consisted of a wooden block with fixed glass slide and a pulley. A pan was attached to another glass slide (movable) with the help of a string. For the determination of Spreadability measured amount of ointment was placed in the fixed glass slide, the movable glass slide with a pan attached to it and was placed over the fixed glass slide, such that the ointment was sandwiched between the two slides for 5 min. The weight was continuously removed now about 50 g of weight was added to the pan. Time taken for the slide to separate was noted. Spreadability was determined using following formula:

$$S=M/T$$

Where S is the spreadability in g/s, M is the mass in grams and T is the time in seconds.

EXTRUDABILITY OF OINTMENT

A simple method was adopted for this study. The formulations were packed in the collapsible tubes following the ointments were lay down in the container. The extrudability of the dissimilar ointment formulations was determined in conditions of weight in grams essential to extrude a 0.5 cm of ribbon of ointment in 10 second.

VISCOSITY OF OINTMENT

Brookfield digital viscometer (in cps) of the prepared ointment formulations as such that is in semisolid state. The spindle T-D (spindle code S94) was rotated at 2.5, 4, 5 and 10 rounds per minute (rpm). The reading, near to 100% torque was noted. Samples were measured at $30\pm1^{o}C$.[14]

ANTI-BACTERIAL ACTIVITY

The antimicrobial activity of various ointment formulations of *Acacia nilotica* bark against *Staphylococcus aureus* and *E. coli* was evaluated by the standard cup plate method and the inhibition zone diameters were measured. Nutrient agar media was used for bacterial culture and incubated at temperature of $37^{o}C \pm 2^{o}C$ for 24 h.

IN VIVO EVALUATION

SKIN IRRITATION STUDY

Three albino rabbits were selected for the study, 24 hours prior to the test, the test sites were depilated on both sides of the spine and demarcated for the application of the formulation.[15] The measured quantity of ointment was applied over the erythema and edema for 48h after application.

STATISTICAL ANALYSIS

The statistical significance of the results were analyzed by one way analysis of variance (ANOVA) followed by Student–Newman–Keul's procedure. Experimental results concerning this study were mean ± (standard error mean) SEM of six parallel observations and $p<0.05$, $p<0.01$ and $p<0.001$ was considered as significant.

RESULT & DISCUSSIONS

PRELIMINARY PHYSICAL ANALYSIS:-

The whole plant material of *Acacia nilotica* extracted with 50% ethanolic solvent. The resultant extract was dried in air until constant weight of the plant extract obtained. The percentage yield of whole plant extractwas found to be 22.08%. The prepared extract was then observed for physical characteristics as mentioned in Table 3.

Table 3. Physical characteristics of 50% ethanolic *A. nilotica* plant extract

Parameters	**Physical properties of *Acacia nilotica extract***
Colour	Dark green
Odour	Characteristic
Taste	Bitter
Consistency	Semisolid
Sense of touch	Sticky
% Yield (w/w)	22.08%

PHYTOCHEMICAL SCREENING:

Identification for various phytochemical constituents was performed with whole plant extract by performing relevant phytochemical tests. The results were depicted as in Table no. 4.

Table 4. Phytochemical evaluation of *A.nilotica*bark extract

Photochemical Test	**Result**
Test For Alkaloids	
Mayer's Reagent	Positive
Dragendroff's Reagent	Positive
Hagers test	Positive
Wagner's test	Positive

Test For Flavonoids	
Lead acetate test	Positive
Ferric chloride test	Positive
Shinoda Test	Positive
Alkaline test	Positive
Test For glycoside	
General test	Positive
Test for Cardiac Glycoside	
Legal's Test (test for cardenoloids)	Positive
Keller killiani's Test (for deoxysugars)	Positive
Liebermann's Test (for bufadenoloids)	Positive
Test for Anthraquinone Glycosides	
Borntrager's Test	Negative
Modified Borntrager's Test	Negative
Test For Carbohydrates	
Molisch test	Positive
Test for pentoses	Positive
Barfoed's Test	Positive
Test For Protein and Amino Acids	
Biuret test	Positive
Million's Test	Positive
Xanthoprotein Test	Positive
Test for proteins containing sulphur	Positive
Test for Triterpenoids and Steroids:	
Salkowaski test	Positive
Libermann-Burchard Test	Positive

IN-VITRO OF PREPARED FORMULATION

a. SPREADABILITY OF PREPARED FORMULATION

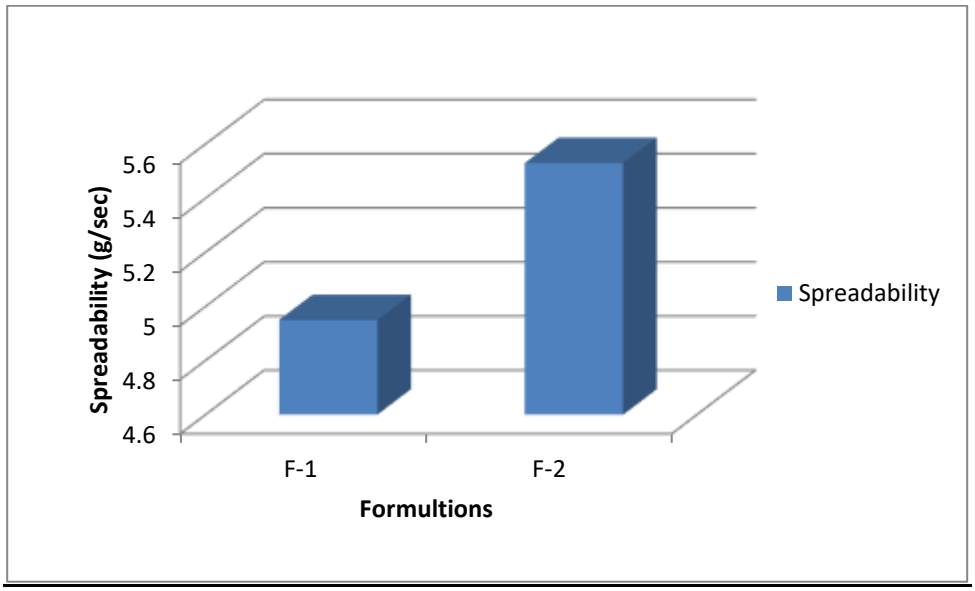

Fig.1: Graph of spreadability for different formulations

b. EXTRUDABILITY OF PREPARED FORMULATION

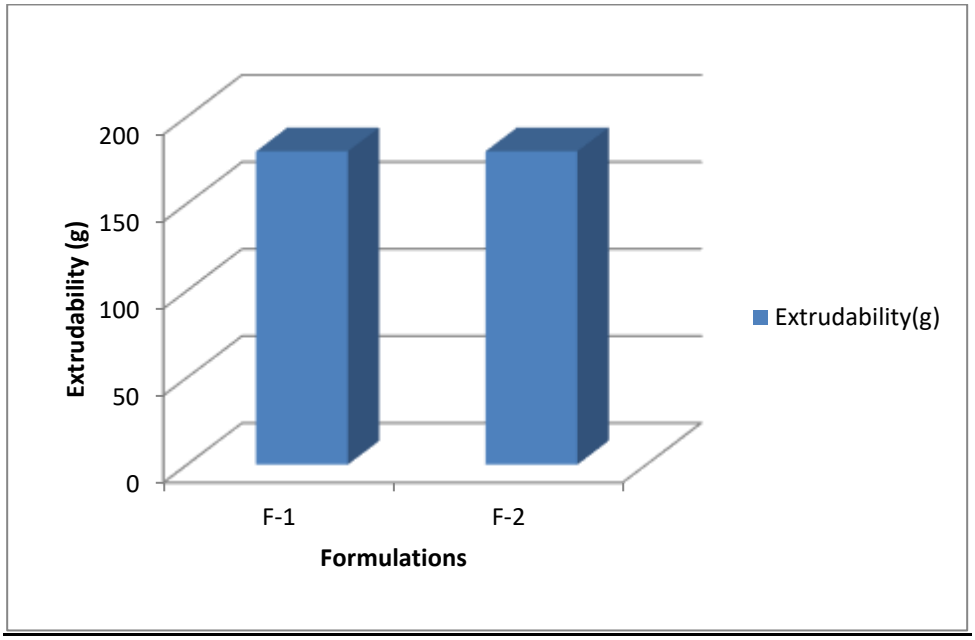

Fig. 2: Graph of extrudability for different formulations

c. VISCOSITY OF PREPARED FORMULATION

Viscosity of ointment

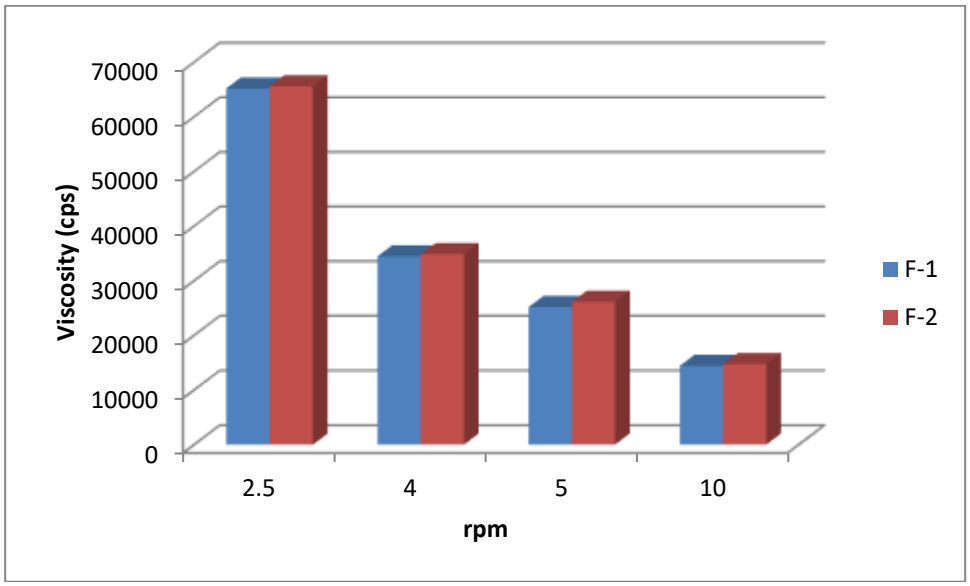

Fig. 3: Graph of viscosity for different preparations Vs rpm

ANTI MICROBIAL STUDY OF PRPARED FORMULATION

Inhibition Zone diameter of different formulations

ZOI = Zone of inhibition

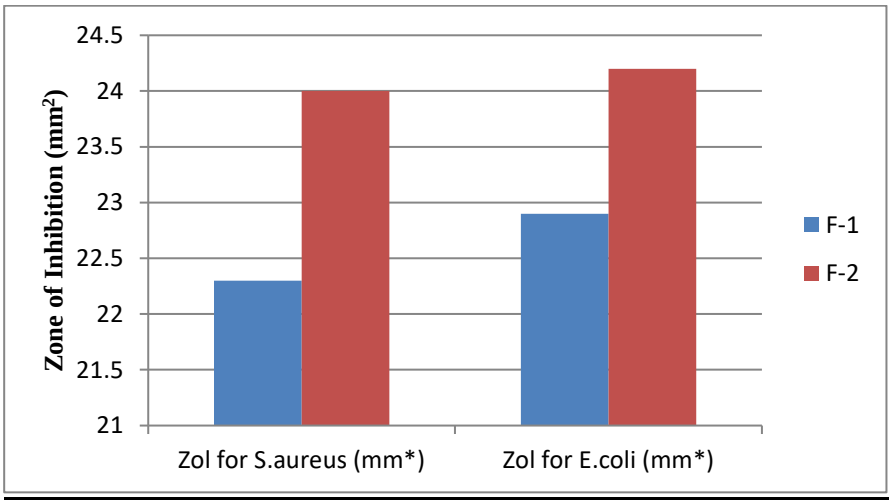

Fig. 4: Graph of Zone of Inhibition for *S.aureus* and *E.coli*

IN VIVO EVALUATION OF PREPARED FORMULATION

a. WOUND CONTRACTION STUDIES:

In the study of the excision wound model, the rate of wound healing was higher. 50% EtOH extract of *A.nilotica* barkshowed significant dose dependent (10% and 20% w/w ointment, topically) wound healing potency. The studies revealed that, when compared to control (4.33±0.31) on day 15, 10% *A.nilotica* bark ointment (1.02±0.13) showed significant improvement ($p<0.001$), while 20% *A.nilotica* bark ointment and standard showed wounds full contraction. It was observed that there is complete healing of the wounded area in different group of animals were clearly visualized in 12 to 15 days of the experimental period.

Table 5. The effect of *A.nilotica bark* ointment on the excised wound in rats

Treatment Groups	Wound Contraction (mm^2) (percentage wound contraction in parenthesis)					
	0 Day	3rd Day	6th Day	9th Day	12th Day	15th Day
A.nilotica bark ointment (10% w/w)	8.26±0.23[a] (0.00)	6.91±0.36[c] (16.34)	5.89±0.13[b] (28.69)	4.11±0.30[b] (50.24)	2.74±0.17[a] (66.83)	1.02±0.13[c] (87.65)
A.nilotica bark ointment (20% w/w)	8.14±0.24[a] (0.00)	6.00±0.37[b] (26.29)	4.79±0.31[c] (41.16)	2.89±0.28[c] (64.50)	1.35±0.20[c] (83.42)	0.23±0.36[c] (97.17)
Betadine ointment (10%w/w)	8.39±0.18[b] (0.00)	5.83±0.36[c] (30.63)	4.81±0.27[c] (42.66)	2.37±0.31[c] (71.75)	1.17±0.32[c] (86.05)	0.09±0.36[c] (98.93)
Blank ointment	8.32±0.39 (0.00)	7.41±0.36 (10.94)	6.99±0.31 (15.99)	6.03±0.24 (27.52)	5.67±0.33 (31.85)	4.33±0.31 (47.95)

Value expressed as mean±SEM; n=6, one way ANOVA followed by Student-Newman-Keuls t-test, t-value denoted significance at a:p<0.05; a:p<0.01; a:p<0.001 respectively.

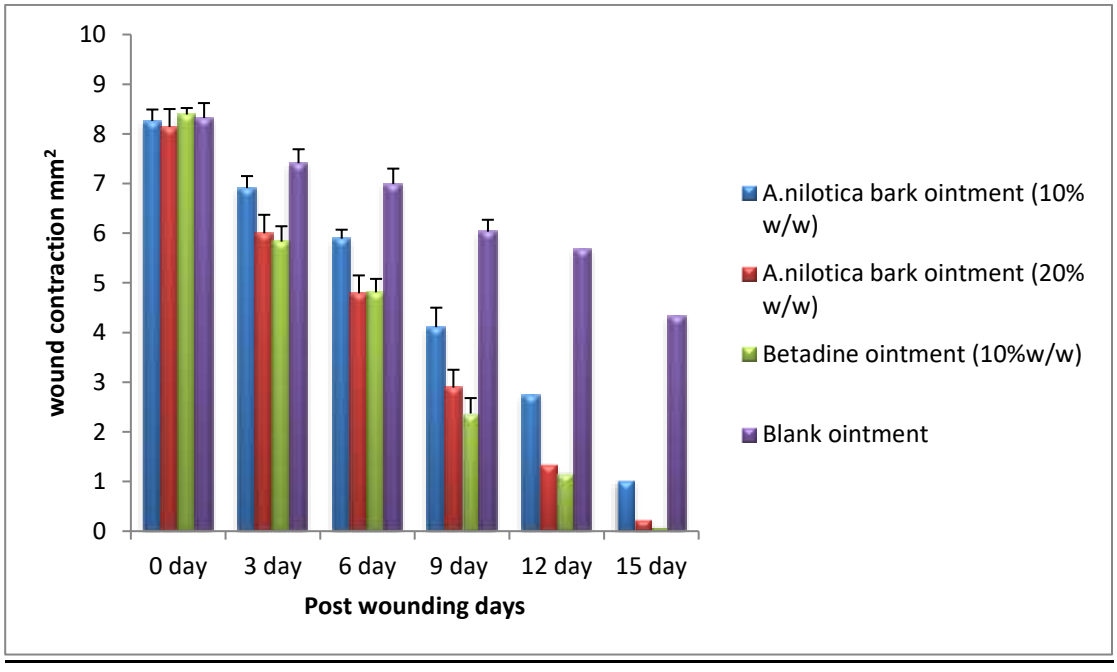

Fig. 5: Graph between Wound contraction Vs Post wounding days

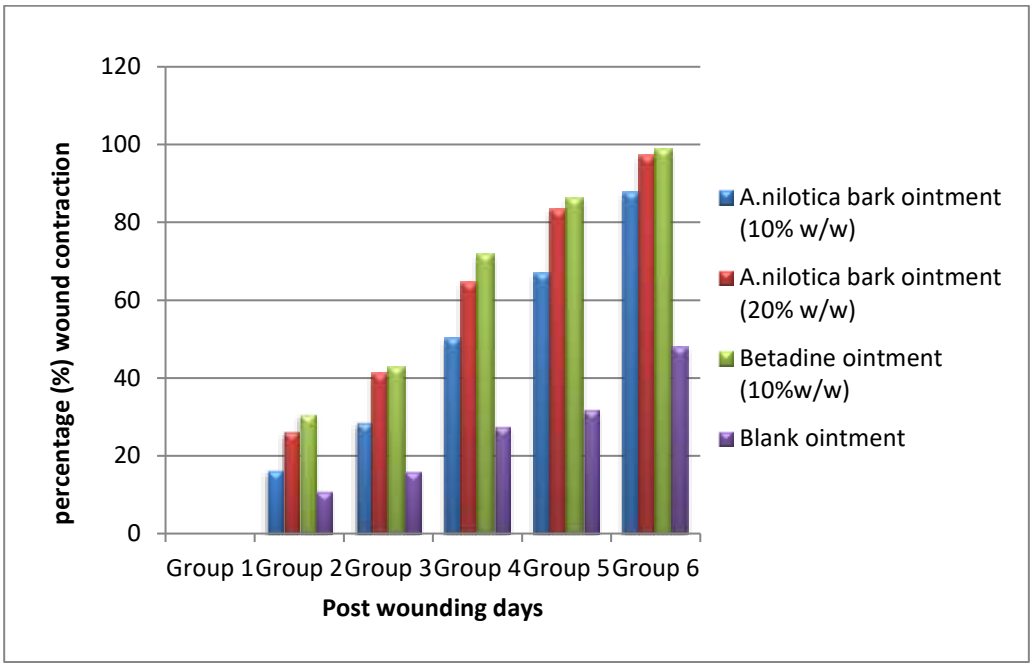

Fig 6. Graph between Percentage wound contraction Vs Post wounding days

DISCUSSION

The prepared formulation of *Acacia nilotica* bark was found to be a good wound healing agent as 20% w/w ointment showed significant contraction of wound as compared to the standard drug Betadine ointment. The percentage wound contraction was found to 98.93% by 20% w/w *A.nilotica* bark ointment. There was on any skin irritation found on application of prepared ointment. The viscosity of prepared formulations was checked at different rpm.

CONCLUSION

Results clearly show that medicated ointment formulation of ethanolic extract of Acacia *nilotica*bark has satisfactory results, pharmaceutically as well as biologically. This prepared extract also promotes fast wound healing than control and non-medicated group. The activity may be mainly due to free radical scavenging and antioxidant activity of the polyphenols present in the bark extract, which are known to reduce lipid peroxidation, an important process for wounds, burns, skin ulcers, thereby reduce cell necrosis.

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