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Wound healing effect of methanolic flower extract of *Bauhinia tomentosa* Linn. with emu oil in rats

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Abstract
Objective: To investigate the wound healing property of methanolic flower extract of *Bauhinia tomentosa* with emu oil.
Method: Male Wister albino rats (n = 25) were used in this study. Excision wounds were created on the skin of five groups of 5 rats using surgical blade under anesthesia. The first group was topically treated with Vaseline alone, group 2 was topically treated with standard i.e., Soframycin ointment, group 3 was treated with Bauhinia flower extract alone, group 4 was treated with Emu oil alone and group 5 was treated with combination of Bauhinia flower extract and Emu oil.
Results: The phytochemical analysis revealed several bioactive constituent including carbohydrates, flavonoids, saponins, tannins & phenolic compounds, proteins and amino acids, gums and mucilage. The extract had significant (ANOVA, $P<0.05$) healing effect on the excision wounds.
Conclusions: These data indicates that *Bauhinia tomentosa* flower extract with emu oil contains potent bioactive compounds containing wound healing activity. *Bauhinia tomentosa* is substantiating its use as a wound healer.

Keywords: Wound healing effect, Bauhinia tomentosa, Emu oil, medicinal plant, collagen tissue, open wound

1. Introduction
A break in the cellular and anatomical architecture of body tissue including the skin, mucus membrane, deep lying tissues or surface of internal organs ranging from incision, laceration, abrasion, puncture and closed wounds such as conclusion, hematoma and crush injuries is termed as wound [1, 2]. It may result from traumatic injuries, metabolic disturbances and long standing debilitating system conditions such as diabetes and hyperglycemia [3-5]. Naturally, wound healing is slow and sometimes may become chronic with a long clinical course there by resulting in a constant release of inflammatory modulators that cause pain and swelling [5]. Chronic wound may become infected with micro- organisms and this may result in delay in the wound healing, septicemia, organ failure and death in severe conditions [6].
Wound healing is an intricate process where the skin or other body tissue repairs and dermis form a protective barrier against the external environment. When the barrier is broken, an orchestrated cascade of biochemical events is quickly set into motion to repair the damage [7]. This process is divided into predictable phases: homeostasis, inflammation, the growth of new tissue (proliferation) and the remodeling of the tissue (maturation), angiogenesis, collagen deposition, granulation tissue formation, epithelialization & wound contraction [7]. The cells include endothelial, fibroblasts, epithelial cells, and myofibroblasts. Several factors contribute to delay in wound healing. These include complications resulting from contaminated infective micro-organisms, anti- inflammatory agents, nutritional deficiencies, inadequate blood supply, and inappropriate movement of the disrupted parts of the body as well as interposed foreign materials such as surgical sutures [8].
Medicinal plants play a prominent role in the new era of modern medicine. Numerous medicinal plants and their formulations are used for various disorders in ethno medical practices as well as in a traditional system of medicines in India. Today's estimates indicates that about 80% of people in developing countries still rely on traditional medicines- based largely on species of plants and animals for their primary healthcare. About 30% of the worldwide sale of drugs based on natural products. India has rich utilizable heritage of local medicinal role not only stored in repositories but also practiced in different parts of the country it is in this context, we find that a number of organizations have started to " back to nature" philosophy and turning towards traditional medical role which is a rich repository of drugs. The growing interest in herbs is of the movement towards changing in the life styles.

This movement is based on the belief that the plants have a vast potential for their use as a curative medicine. Natural products are an integral part of human health is system at present; there are now popular concerns over toxicity and side effects of modern drugs. There is also a realization that natural medicine is safer.

Some plants contain phytochemicals that have effects on the body. Herbs have long been used as the basis of traditional Chinese herbal medicine, with usage dating as far back as the first century and far before. In India, the ayurveda medical system is based on herbs medical use of herbs in western cultures has its roots in the Hippocratic elemental healing system, based on a quaternary elemental healing metaphor. Modern pharmaceuticals had their origins in the crude herbal medicines, and to this day, some drugs are still extracted as fractionate/ isolate compounds from raw herbs and then purified to meet pharmaceutical standards [9].

Bauhinia tomentosa also known as yellow bauhinia, mountain ebony, purple orchid, kachnar. It belongs to the family fabaceae. It is native to tropical Africa & can be found in India & Srilanka. It grows upto 6-12m height, branches spreads 3-6m, leaves are lobbed and usually 10-15 cm across and divided into two lobes, light green in color with a leathery texture. It produces, large bell shaped bright yellow flowers with a black to deep maroon colored centre from December to march. The fruits are pea like slender and velvety [10]. The active constituents of bauhinia flower are flavonoids such as isoquercitrin, rutin & traces of quercetin. Seeds yield proteins, mucilage and saponins [11]. The literature reported that the activities like anti-bacterial activity [12], anti-hyperglycemic and anti lipidemic activity [13], anti-diabetic activity [14], anti-ulcer activity [15], stimulation of immune system and scavenging of free radical generation [16]has been carried out.

Essential fatty acids are the fatty acids which humans ingest because the body requires for good health but cannot synthesize them [17]. There are two families of essential fatty acids: omega-3 (n−3) and omega-6 (n−6) fatty acids. As these fatty acids are not saturated with hydrogen (H) atoms (and contain more than one double bond between the atoms) they are called ' polyunsaturated fatty acids ' (PUFAs). Most PUFAs are of plant and fatty fish origin.

There are three major types of ω-3 fatty acids that are ingested in food and utilized by the body: Alpha Linolenic Acid (ALA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) [18]. EFAs are required for the proper structure and function of every cell in the body, and are important for optimal health. EFAs increase the absorption of vitamins and minerals; nourish the skin, hair and nails; promote proper nerve functioning; help produce hormones; ensure normal growth and development; and prevent and treat disease [19].

The natural absorption promoters documented so far include essential oils, terpenes, terpenoids, fatty acid esters, fatty acid glycols, and herbal extracts. The essential oils are nontoxic, non-allergic, and compatible with drug and excipients have received much attention of researchers and found one of the promising groups of candidates to be employed as clinically acceptable penetration enhancers. Essential oils present a large range of chemically acceptable and relatively safe penetration enhancers to aid percutaneous drug delivery and are considered as GRAS (generally regarded as safe) compounds for medicinal use. They have been reported to use for permeation enhancement of both hydrophilic and lipophilic drugs. They cause no skin toxicity or if any, only mild irritation [20].

Emu oil is a bright yellow liquid [21]. It contains 98.8% and 98% of the average amount of lipids in oils obtained from subcutaneous and retroperitoneal adipose tissues respectively. The fatty acid composition of emu oil depends on the diet of the bird, the method of extraction and the type of adipose tissue from which the oil is extracted [22, 23]. Emu oil is reported to contain all the three omega fatty acids (i.e., ω-9,ω-6 and ω-3), making it an excellent supplement. The largest component is oleic acid, a mono-unsaturated ω-9 fatty acid (18:1), comprising > 49.1% of the total fatty acids. Emu oil also contains unsaturated fatty acids like 9.5% linoleic acid (18:2, ω-6), 1.1% α-Linolenic acid (18: ω-3), and 32.5% saturated fatty acids as previously reported [24]. The non-glyceride (TG) fractions include various compounds like anti-oxidants, notably carotenoids, flavones, polyphenols and phospholipids [25].

2. Materials and Methods

2.1 Plant Extraction

The flowers of *Bauhinia tomentosa* were collected from local area of Mangalagiri, Guntur and shade dried. Then the dried flowers were made into powder. The powder was extracted using methanol as solvent by soxhlet process. The extract was subjected to phytochemical screening for the presence of various constituents.

2.2 Formulation

The methanolic flower extract of bauhinia (2gm) was mixed with the Emu oil (2ml) and it was formulated as a Liniment. As the fixed oils are more suitable as Liniments.

2.3 Animal Experimentation

The experimental protocols were approved by the ethical committee of animal care and use. All the chemicals and solvents used in this study were of standard and analytical grade and solutions were freshly prepared prior to experimentation.

2.3.1 Preparation of animals

Animals were kept for one week to acclimatize to laboratory conditions before starting the experiment, they were allowed to free access of water and standard rat feed-libitium pellets.

2.3.2 Groups

The male Wister albino rats weighing (150-250gms) were used for the study. Animals were divided in to five groups each group consists of 5 animals.

Table 1

S. NO	Groups	Treatment
1	Group I	Control (Vaseline)
2	Group II	Standard (Soframycin Ointment)
3	Group III	Test 1 (Bauhinia flower extract)
4	Group IV	Test 2 (Emu oil)
5	Group V	Test 3(Bauhinia flower extract with Emu oil)

2.3.2 Acute toxicity tests

Prior to experimentation, the lethal dose 50 (LD_{50}) of the extract on the test of animals was determined by giving the increased concentrations of 1, 2,3,4,5 mg/kg BW of liniment. Continual monitoring of animals was done for mortality and non-mortality.

2.3.3 Excission Wound Model

- Wister male albino rat weighing between 150-250gms

body weigh were selected

- The rats inflicted with Excision (open) wounds as described by Morton and Malone anaesthetized prior to creation of the wounds.
- The dorsal fur of the animals was removed by using a suitable depilatory (Fem) cream.
- The rats were divided into 5 groups, followed as mentioned above.
- The wound to be created was outlined on the back of the animals with stainless steel stencil.
- A full thickness of the Excission wound of 2 cms width and 0.2 cms depths was created along the marking using toothed forceps, pointed scissors.
- The entire wound was left open.
- The animals were treated respectively for 21 days.
- The measurement areas of the excision wound model taken on 1st, 2nd,3rd,4th,8th,12th,16th and 21st day.
- The wounds were traced on 1mm² graph paper on the wounding and subsequently on alternate days until healing was completed.
- The period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound.
- In the excision wound model, granulation tissue formed on the wound was excised on the 22nd postoperative day and weight is recorded.
- The tissue was dried in oven at 60 ℃ and the dry weight was again noted.
- The protein content of the tissue extract was also estimated.
- Acid hydro lysate of the dry tissue was determined using hydroxy proline.

2.4 Estimation of hydroxy proline

Granulation tissue (wound area) from both control and treated rats was collected and then dried in a hot air oven at 60 ˚C for 12 hours and weighed to determine the dry granulation tissue. Dried granulation tissues were added with 6N hydrochloric acid (1.0 ml for 100 mg of sample) and then kept at 110 ˚C for 24 hours in sealed tubes. 1.0 ml of acid hydrolysate sample was mixed with 1ml each of 0.01 M Copper Sulphate solution, 2.5N Sodium Hydroxide and 6% Hydrogen Peroxide. The solutions are mixed and incubated at 80 ˚C for 5 minutes. The tubes are chilled in an ice water bath and 4ml of 3N Sulphuric acid was added with agitation followed by addition of 2 ml of P-DAB (5%) solution and mixed thoroughly. The tubes were placed in a water bath at 70 ˚C for 15 minutes and then chilled in an ice water bath. The absorbance was measured at 540 nm [26, 27].

2.5 Statistical Analysis

The statistical analysis was done using one- way Analysis Of Variance (ANOVA). All data were reported as mean±SEM with level of significance of $P< 0.05$.

3. Results

3.1 Phytochemical analysis of the *B. Tomentosa* flower extract:

The percentage yield of the flower extract was 34.4%. The phytochemical analysis of the leaf extracts revealed number of relative amount of bioactive and amino acids, gums and mucilage. Constituents including carbohydrates, flavonoids, saponins, tannins, phenolics compound, proteins.

Table 2

Test For Extracts	Name of the Test
Carbohydrates	Molisch's test
Flavonoids	Magnesium turning's test
Saponins	Foam test
Tannins and Phenolic compounds	Dil. ferric chloride
Proteins and amino acids	Biuret test
Gums and mucilage	Molisch's test

3.2. Acute toxicity test

There was no mortality in the first as well as in the second stage of acute toxicity test. All the animals were exposed to increasing concentrations of the leaf extract up to highest concentration of 5000 mg/kg BW used in this lethality test were survived. Therefore, the LD_{50} of the extract was not deduced.

3.3. Wound healing effect of the methanolic flower extract of *B. tomentosa* with emu oil

In group of animals, that had excision wounds were topically treated with petroleum jelly alone, healing occurred but at a slow rate through the first, second and third treatment regimens. Topical treatment of the excision wound with 2gm/ 2ml lotion resulted in wound healing and a significant ($P<0.05$) reduction in the wound area from the second treatment regimen compared with the negative control.

Treatment with *B. tomentosa* flower extract incorporated in emu oil showed significant ($P<0.05$) wound healing activity in all the concentrations used in the study. Increased concentrations of the extract resulted a significant ($P<0.05$) wound healing effect on the excision wound.

3.4 Results

Excission Wound Model

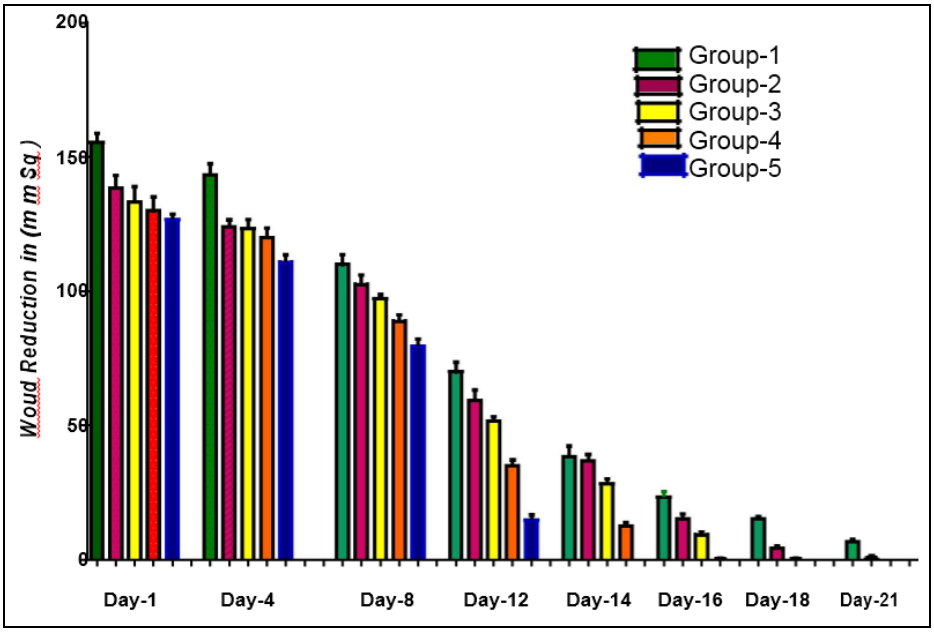
Table 3

S. No	Groups	Wound Contraction in mm²						
		1st day	8th day	12th day	14th day	16th day	18th day	21st day
1	Control	155.5±3.304	110±3.651	70±3.651	41.63±4.014	23.33±2.108	25.33±0.8819	6.667±1.054
2	Standard	138.3±4.773*	102.5±3.594*	59.33±3.930*	36.83±2.455*	15.17±1.833*	4.337±0.6667*	0.8667±0.5327
3	Test-I	133.3±5.578**	97.33±1.453**	51.63±1.606**	28.33±1.801**	9.333±0.8819**	0.4600±0.1558*	0.0333±0.02108
4	Test-II	130.0±5.167***	88.33±2.372***	35.00±2.129***	12.50±1.384***	0.2833±0.0945***	0.07667±0.O166*	O.OOO
5	Test-III	126.7±2.108***	79.56±2.754***	14.83±1.922***	0.25±0.1566***	0.0333±0.0210***	0.0112±0.0012*	0.0000

Each Value Is Represented As Mean±SEM, No Of Animals=6

(***) P values was expressed in <0.01 when compared to control

3.5 Rate of Contraction



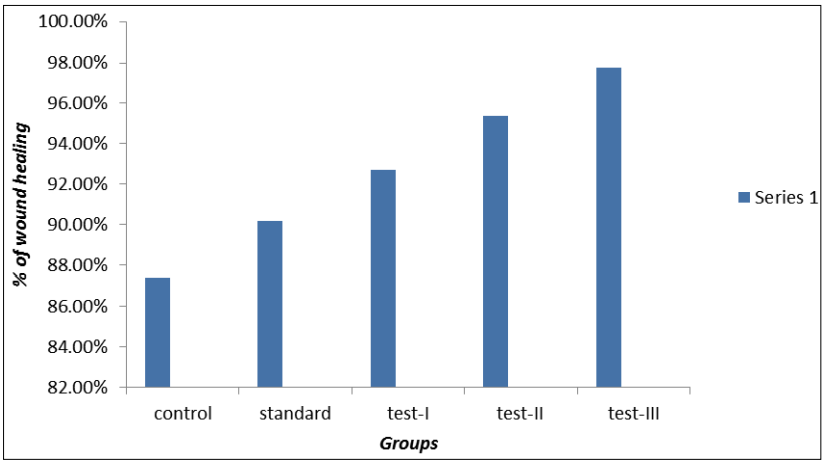
3.6 Days

Group-I: Control (Treated With Vaseline)
Group-II: Standard (Treated With Soframycin ointment)
Group-III: Test-I (Treated With Bauhinia Flower Extract)
Group-IV: Test-II (Treated With Emu Oil)
Group-V: Test-III (Treated With Emu Oil & Bauhinia Flower Extract)

Table 4

S. No	Group	Dose/Kg	% Wound Healing
1	Control	1.5%W/W	87.4%
2	Standard	1.5%W/W	90.2%
3	Test-I	1.5%W/W	92.72%
4	Test-II	1.5%W/W	95.37%
5	Test-III	1.5%W/W	97.75%

$$\%\ of\ wound\ closure = \frac{Initial\ area\ of\ wound - N^{th}\ day\ area\ of\ wound}{Initial\ area\ of\ wound} \times 100$$

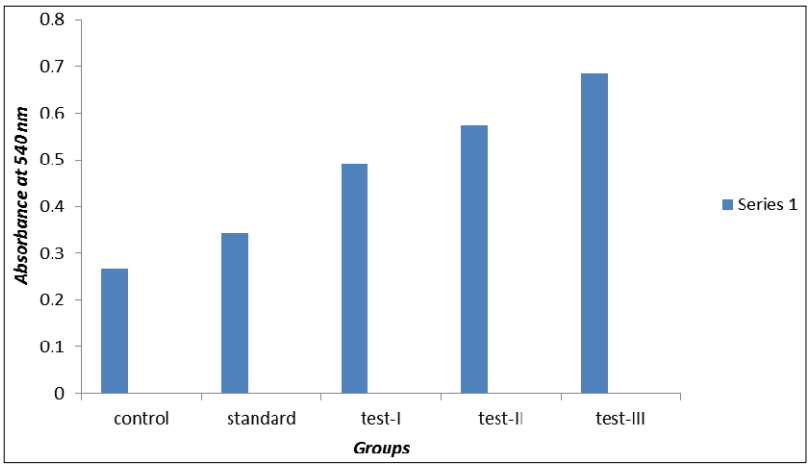


3.7 Biochemical Parameters

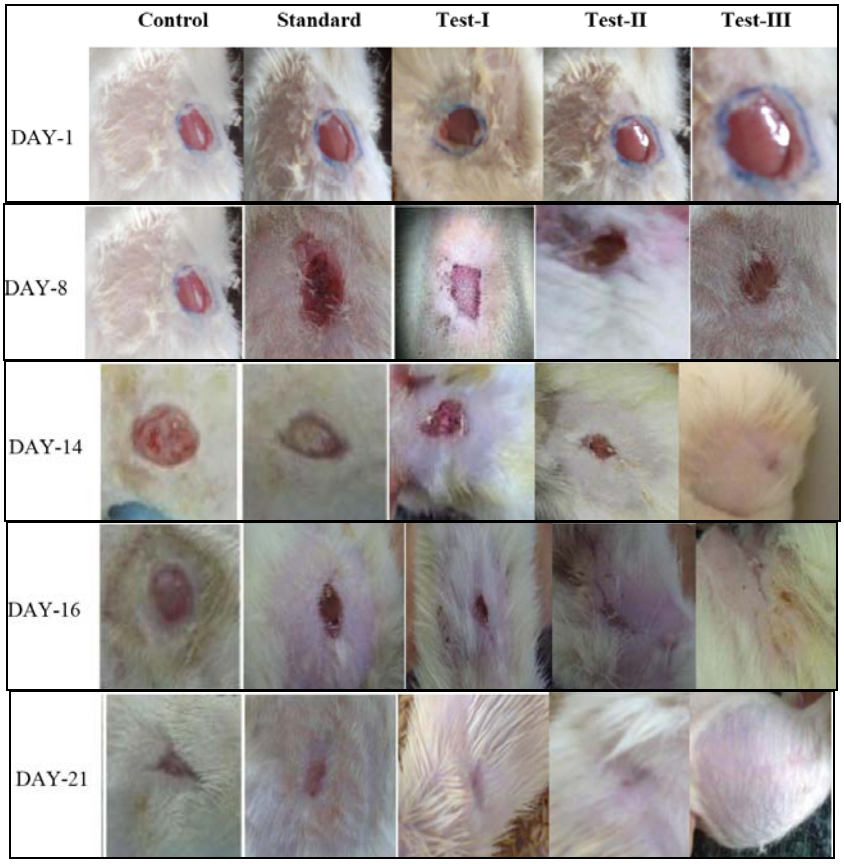
Table 5

S. No	Parameters	Control	Standard	Test-I	Test-II	Test-III
1.	Hydroxy Proline	0.2667±0.02186	0.3417±0.01641	0.4933±0.02848	0.5733±0.03518	0.6850±0.0334

Each value is represented as Mean±SEM No of Animals=6



Excission Wound Photos



4. Discussion

Since past few years Emu production is becoming a developing trend in industries in India has relied largely on breeding and selling chicks into an expanding with evidences for therapeutic efficacy of the fixed oil extracted from fat of the Emu bird for its applications in relieving joint injury pains, muscle injury pain, anti-inflammatory, anti- microbial and especially in offering unsurpassed penetration and also the emu oil evidences in application in cosmetic preparation from USA and Australia.

To date the evidence for the efficacy of emu oil enhancing the therapeutic effect of an herbal drug in wound healing, the work was carried out using one model.

4.1 Bio efficiency of Emu oil on wound model

Wound healing is a complex and dynamic process of restoring cellular structure and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage where by the area of the wound undergoes shrinkage. It has 3 phases; inflammatory, proliferative and maturational and is dependent upon the type and extent of damage, the general state of the host's health and the ability of the tissue to repair. The inflammatory phase is characterized by homeostasis and inflammatory, followed by epithelialization, angiogenesis, granulation tissue formation and collagen deposition in the proliferative phase. In the maturational phase, the final phase of wound healing undergoes contraction resulting in a smaller amount of apparent scar tissue.

In our study screening of wound healing activity, we have used one model to assess the effect of emu oil with ethanolic

extract of *Bauhinia tomentosa* Linn. Flower on various phases of wound healing supporting with the biochemical supporting modification.

The present study was undertaken to evaluate whether the emu oil enhances the effect of an herbal extract or itself has effect in promoting wound healing in experimentally induced wounds in rats. The lotion containing ethanolic extract of *Bauhinia tomentosa* Linn. Flower and emu oil was found to promote the wound contraction and period of epithelialization; collagen synthesis was stimulated compared to the groups that received the emu oil only, herbal extract only and standard ointment.

4.2 Excission wound model

Granulating tissue consists of a combination of cellular elements, including fibroblasts and inflammatory cells, along with new capillaries embedded in a loose extracellular matrix of collagen, fibronectin and hyaluronic acid. In the present study wound healing was evaluated by quantitative measurements of collagen and hexosamine content of the granulation tissues, in addition to the measurements of wound contraction and epithelialization. Both area measurement and biochemical finding indicates that significant improvement occurred in the healing process following treatment with lotion containing both emu oil and herbal extract compared to other groups. The results of the study show that the production of collagen by herbal extract in open wounds can be modulated by enhanced effect of emu oil.

This study showed that the emu oil is reported to have anti-inflammatory activity and proliferative action. It was observed that there was increase in the rate of wound contraction and complete skin formation compared to other groups. This demonstrated by increased hydroxyl proline content, which is a reflection of increased collagen levels, which indicates better maturation and proliferation of collagen by increased collagen orientation.

5. Conclusion

The biochemical analysis and wound area measurements results from this study clearly indicate that the emu oil can enhance the efficacy of a herbal drug and is beneficial in promoting wound healing with new skin.

This work may promote ideas for a future research scientist or an industrialist to work on emu oil based formulations to bring a drug formulation with good therapeutic efficacy with no side effects i.e. skin allergies to a man kind in the pharma market.

In our work wound healing results reveals the truth of Emu oil having good transdermal activity and enhances the penetration of ethanolic extract of *Bauhinia tomentosa* Linn. flower in promoting the healing process of open wound model and the Emu oil with herbal extract showed more significant results when compared to standard i.e. soframycin ointment.

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