EVALUATION OF WOUND HEALING EFFECT OF AQUEOUS LEAVES EXTRACT OF *Smilax china linn.,* ON WISTAR RATS

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The crucial role of medicinal plants in addressing diverse health issues is irreplaceable. *Smilax china linn.,* is one of the medicinal plants used traditionally for treatment of wounds. However, there were no scientific reports documented so far on the wound healing activities of this plant leaves aqueous extract, the present study was designed to explore the same. Thus, the present study provides a scientific evaluation for the wound healing potential of the crude aqueous extract of *Smilax china linn.,* leaves. *Methods*. The crude extraction was carried out using water. The crude extract was prepared in 0.5% (w/w) ointment and evaluated for wound healing activity using excision and incision wound models in Swiss albino mice. *Results*. The aq.extract of *smilax china linn.,* 0.5% ointments significantly reduced period of epithelialization and increased wound contraction rate and tensile strength compared to the negative control group ($P < 0.05$). The wound healing activity of 0.5% (w/w) ointment treated group was showing good result than povidone ointment treated groups in excision wound model. *Conclusion*. These results demonstrate that the crude extract of *Smilax china linn.,* leaves possesses wound healing activities. *Smilax china linn.,* accelerated wound healing in rats, thus supporting its traditional use. This justifies the traditional claimed use of the plant for treating uninfected and wounds caused by excision wound model.

Key words: Excision wound model, *smilax china.,*,wound healing activity

Introduction:

The traditional systems of Indian medicine especially ayurveda, siddha and unani employed a large number of medicinal plants for treatment of skin diseases which includes cuts, wounds and burns[1]. For centuries, medicinal plants have been employed to treat a variety of skin issues, including cuts, wounds, and burns. Skin is the largest organ in the body and covers the body's entire external surface[2]. Initially, inflammation triggers the body's response to injury. Wound healing involves three stages: inflammation, proliferation, and remodeling. Begins immediately after an injury to the epidermal layer and might take years[3]. Dynamic process including highly organized cellular, humoral, and molecular mechanisms. Has 3 overlapping phases which are inflammation, proliferation, and remodelling. Any disruption leads to abnormal wound healing[4].

Plants and their derivatives have long been used for wound healing due to their potential therapeutic activity[5]. Key bioactive compounds found in medicinal plants include alkaloids, tannins, flavonoids, and phenolic compounds[6]. These plants, often utilized as spices and food, hold significant importance in traditional medicine. Smilax China Linn., (family: smilacaceae)is a china traditional plant which having more therapeutic activities[7]. A large number of plants are used by traditional medical practitioners in many countries for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms[8]. smilax china linn., commonly known as china root, ayadi and kondadantena[9].

Fresh leaves of Smilax China Linn., were collected in Sir.C.R.Reddy college of pharmaceutical sciences in Eluru, west godavari district, Andhra pradesh, India. In july and authenticated by department of botony, Acharya Nagarjuna university, guntur, India. A herbarium is maintained in Sir.C.R.Reddy college of pharmaceutical sciences, Eluru, Andhra Pradesh , India. The plant Smilax China

Linn., (family: smilacaceae) are proved to shown the Psoriatic Arthritis[10], hepatocellular carcinoma[11] by inhibiting glycolysis, HFHS-induced inflammation[12] by regulating the gut-liver axis, anti-pelvic inflammation[13], Anti-metastatic effect[14], Tumoral cytotoxic[16], inhibits migration[17], Antiviral and anti-proliferative[18] glycoproteins and antioxidative[19] . A survey of literature revealed that no systematic approach has been made to study wound healing activity of this plant. The leaves of Smilax China Linn., contains Rutin, bismilachinone, Kaempferol[22], Kaemperide, Morin, Kaempferol 7-O-α-L-ranmnoside, Kaemperin, rutin, engeletin, isoengeletin, smiglaside E[23], heloniosides B and smilachinin[24]. This study aims to address the global need for safer and more effective wound-healing agents. It seeks to bridge the gap between ancient literature's claims and modern scientific evidence, striving to discover agents that can promote healing. By doing so, the study aims to reduce side effects in patients and prevent severe complications such as amputation, ultimately benefiting patients worldwide. In this study we assess the wound healing ability based on chemical constituent therapeutic activities of the leaves of Smilax China Linn.,

Materials and method

Collection of Plants and Authentication:

The collected plant material has authenticated by botany S. Prasad rao , Research Officer , Survey of Medicinal Plants Unit, Sir C. R. Reddy autonomous College, Eluru. A voucher specimen (Voucher No. ATC28/05/2023) has been deposited at the herbarium unit of the Department of Botany, Sir C. R. Reddy autonomous College, Eluru , Eluru (District), Andhra Pradesh, India.

Chemicals : The chemicals ether, surgical spirit were purchased from Mahalasa pharma , goa, india and Povidone iodine 5% ointment.

Preparation of Extract:

Dried leaves powder (500 gm) of plant was extracted with water using soxhlet apparatus for 48 hours. Extracts were concentrated by rotary vaccum evaporator under reduced pressure. Percentage yield of extract was calculated on air dried basis.

The dried extract was evaluated for the presence of various phytoconstituents using standard procedures.

Preliminary phytochemical tests for identification of plant constituents The aqueous extract of Stem of Smilax china was subjected to preliminary phytochemical screening for detection of various plant constituents[25].

Tests for carbohydrates :

Molisch's test :

To the filtrate solution add few drops of alcoholic alpha-naphthol solution, shaken and add concentrated sulphuric acid from sides of test tube formation of violet ring at the junction of two liquids indicates the presence of carbohydrates .

Benedict's test :

Equal volume of benedicts reagent and filtrate solution in test tube were mixed. Then heated in boiling water for 5mins. Appearance of yellow, green ,or red solution may indicates the presence of reducing sugars.

Tests for proteins :

Biuret test :

To the 3ml of filtrate solution add 4% of sodium hydroxide and add few drops of 1% copper sulphate solution. Formation of violet or pink colour indicates the presence of proteins.

Xantho proteins test :

To 3ml of filtrate solution add 1ml of concentrated sulphuric acid. formation of white precipitate indicates the presence of proteins.

Tests for amino acids :

Ninhydrins test :

Heat 3ml of filtrate solution and add 3 drops of 5% ninhydrin's solution in boiling water bath for 10 mins. Formation of purple or bluish colour indicates the presence of aminoacids.

Millons test :

Heat 3ml of filtrate solution with millons reagent formation of dark red colour indicates the presence of amino acids.

Test for fats and oils :

Sudan iii test :

Place a drop of filtrate solution on glass slide. Add sudan red III reagent. After 2 min wash nwith 50 % alcohol. Mount in glycerine and observe under microscope. Formation of red oil globules indicates the presence of fats and oils[26].

Tests for alkaloids :

To the filtrate solution add dil,HCl shake well and filter with the filtrate perform the following

Dragendroff's test :

To 2-3ml of filtrate add few drops of dragendroff reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

Hagers test :

To 2-3ml of filtrate add few drops of hagers reagent. formation of yellow precipitate indicates the presence of alkaloids.

Tests for steroid :

Salkowski reaction test :

To the filtrate add 2ml of chloroform and 2ml of concentrated sulphuric acid and shake well. Chloroform layer doesn't appears as red and acid layer shows greenish yellow fluorescence indicates the presence of absence of steroids.

Libermanns test :

Mix 3ml of filtrate solution with 3ml of acetic anhydride. Blue colour doesn't appears indicates the absence of steroids.

Test for flavonoids :

Shinoda test :

To the filtrate add 5ml of 95% ethanol, few drops of concentrated Hcl and 0.5g of magnesium turnings. Pink colour is not observed. Indicates the absence of flavonoids .

Lead acetate test :

To the small quantity of filtrate add lead acetate solution. Yellow colour precipitate is not formed indicates the absence of flavonoids.

Test for glycosides :

Keller killani test :

To the 2ml of filtrate solution add glacial acetic acid, one drop of 5% ferric chloride and concentrated sulphuric acid. Appearance of reddish brown colour at the junction of two liquid layers and upper layer appears as bluish green. Indicates the presence of glycosides[27].

Legal test :

To the filtrate solution add 1ml of pyridine ,1ml of sodium nitroprusside. Formation of pink to red colour indicates the presence of glycosides.

Tests for tannins and phenolic compounds :

Ferric chloride test :

To the filtrate add 5% ferric chloride solution . formation of deep blue black colour indicates the presence of tannins/phenolic compounds.

Bromine water test :

To the filtrate solution add bromine water. Discolouration of bromine water indicates the presence of tannins/phenolic compounds[28] .

Table no. 1

S.no	Phytochemical constituents	Smilax china linn., aq. extract
1.	Proteins	+ve
2.	Aminoacids	+ve
3.	Steroids	-ve
4.	Tannins/phenol	+ve
5.	Fats and oils	-Ve
6.	Flavonoids	+ve
7.	Glycosides	+ve
8.	Saponins	-Ve
9.	Alkaloids	+ve
10.	Carbohydrates	-Ve
11.	Gums and mucilage	-Ve

Preparation of ointment formulation of aq. extract of smilax china

In the pharmaceutical industry, ointments are manufactured by melting oil and aqueous phases in two separate jacketed vessels with agitators for proper mixing. The two phases are transferred to the main ointment vessel through valves and pipes. The additional stirrers in the main vessel provide agitation. During the entire course of operation, uniform mixture of all the components (base + drug) is crucial[29].

Method of preparation:

- By using fusion method ointment formulation is done, one by one all the ingredients are melted according to their melting points. After melting the product is cooled in water bath. Finally the extract was incorporated.
- From the above ointment base 30 mg was taken and to that 500 mg of leaf extract is mixed and labeled as 500mg concentration[30].

Table No : 2

Formulation of ointment ingredient list

S.NO	NAME OF INGREDIENT	F_1 100gm	F_2 100gm	F_3 100gm
1.	Smilax china aq. extract	200mg	500mg	1000mg
2.	Wool fat	5 gm	5 gm	5 gm
3.	Cetostearyl alcohol	5 gm	5 gm	5 gm
4.	Hard paraffin	5 gm	5 gm	5 gm
5.	Yellow soft paraffin	85 gm	85 gm	85 gm

Evaluation of ointment

All the prepared ointments were characterized for the parameters such as appearance, odor, color, homogeneity, pH, spreadability, hardness, water number, and viscosity measurements.

Organoleptic characteristics

All blank formulations (i.e., formulations without active ingredient) and drug-loaded formulation were tested for physical appearance, color, texture, phase separation, and homogeneity. These characteristics were evaluated by visual observation. Homogeneity and texture were tested by pressing a small quantity of the formulated cream and gels between the thumb and index finger. The consistency of the

formulations and the presence of coarse particles were used to evaluate the texture and homogeneity of the formulations. Immediate skin feel (including stiffness, grittiness, and greasiness) was also evaluated[31].

PH

About 2.5 g of all formulations were taken in dry beaker and 50 ml of water was added. Beaker containing ointments was heated on water bath at 60–70°C. The pH of ointments determined using a pH meter (pH Tutor, Eutech Instruments). The determinations were carried out in triplicate and the averages of three readings were noted.

Spreadability :

Spreadability of the formulation was determined by an apparatus suggested by Multimer with some modifications [15]. It consists of a wooden block having a pulley at one end with fixed glass slide on block. An excess of ointment (3 g) placed on ground plate. The ointment was sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. A 1 kg weight was placed on the top of the two plates for 5 min to expel air and to provide a uniform film of the ointment between the plates. Excess of ointment was scrapped off from the edges. The top plate was then subjected to pull of 240 g. With the help of spring attached to the hook and time required by the top plate to cover a distance of 10 cm was noted. A shorter interval indicates better spreadability[32].

Spreadability was calculated using the following formula:

$S = M \times L/T$

Where, S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide and

T = Time (in seconds) taken to separate the slide completely each other.

Viscosity:

Brookfield Synchro-Lectric Viscometer (Model RVT) with Helipath Stand was used for rheological studies. The sample (50 g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a T-D spindle at 10, 20, 30, 50, 60, and 100 rpm. At each speed, the corresponding dial reading on the viscometer was noted. The spindle speed was successively lowered and the corresponding dial reading was noted. The measurements were carried in triplicate at ambient temperature. Direct multiplication of the dial readings with factors given in the Brookfield Viscometer catalog gave the viscosity in centipoises (CPS)[33].

Water number:

Water number is the maximum amount of water that can be added to 100 g of base at a given temperature. It was determined by continuously stirring the base with the addition of distilled water. When no more water was absorbed into the base evidenced by droplets of water, remaining in the container was taken as end point.

Hardness/strength:

Hardness of formulations was determined using Texture Pro CT V1.3 (Build 15 Brookfield Engineering Labs, Inc.) texture analyzer. It is based on the speed of the displacement of probe into sample (ointment) at a given distance. The probe was moving down at a speed of 2 mm/s till a 7 g surface trigger was attained. At this point, the probe was in full contact with the sample surface. Then, the probe continued to penetrate to a depth of 4 mm at a speed of 2 mm/s. At this point, the probe returned to its starting position. The penetration depth of a standard 4 mm needle (P/2N) at a constant 10 kg load force was measured to represent the hardness of the formulation. The peak load (maximum force) was registered and is considered a measure of firmness of the product – the bigger the force the thicker/harder is the sample[37]. All tests were

conducted 2 times at room temperature (25±2°C) and values of peak force were expressed in g.

Drug content:

Content of salicylic acid in the formulation was determined by diluting 1 g of ointment equivalent to 2 mg of drug in 10 ml of ethanol and volume was made up to 100 ml with pH 7.4 phosphate buffer[34]. Absorbance was measured at 275 nm using ultraviolet (UV)-visible spectrophotometer and percentage drug content was calculated and average of three determinations[35] was noted (n=3).

Table no: 3

Phytochemical evaluation of ointment formulations

Formulation code	Physical appearance	texture	Phase separation	Homogeneity	Immediate skin feel
F1	Opaque	Rough and hard	No	Homogeneous	No grittiness or greasiness
F2	Opaque	Smooth	No	Homogeneous	Little grittiness was observed and no greasiness
F3	Opaque	Smooth	No	Homogeneous	No grittiness or greasiness

Table no: 4

Evaluation parameters of ointment formulations

Formulation code	pH(mean-+Sd)#	Viscosity at 10 rpm(cps) (mean+-sd)#	Spreadability g.cm/s (mean+-sd)#	Hardness (g) (mean+-sd)#	Water number (mean+-sd)#	Drug content(%)
F1	6080 ±0.152	2851±9.93	80.00±3.83	243 ±4.13	1.2±025	-

F2	7.01±01.85	2613±8.13	109.09±5.13	220 ±3.90	1.3±008	-
F3	6.90±0.189	2472±7.23	112.57±4.23	157 ±3.83	1.3 ±0.12	-

Animals:

Male *Wistar* rats (220-260 g) were used for all experiments. Animals were housed under conditions of controlled temperature (25±1 ºC) with relative humidity of 45-55% and lighting (lights on: 6 am to 6 pm) and had free access to food and water and the animals were acclimatized for a period of dark/light cycle. The animals were acclimatized for a period of two weeks and were kept under pathogen free conditions[36].

Experimental protocol:

Animals were selected, weighed (200–250g) and devided into five groups (n=6), namely standara, control, two groups belonging to aqueous extract ointment and lepam of *smilax china* linn.,

Groups of six animals in each groups as follows and the treatment was done topically in all the cases[37]:

Group I- Normal animals : Did not received injury for wound formation

Group II- Control animals: Received injury for wound formation but did not receive any ointment or drug treatment

Group III- Drug treated animals: received injury for wound formation and treatment with *smilax china*., leaves aqueous extract ointment (given 500 mg/kg)

Group IV- Drug treated animals: received injury for wound formation and treatment with *smilax china*., leaves aqueous extract lepam (given 600 mg/kg)

Group V- Drug treated animals: received injury for wound formation and treatment with povidone iodine 5% ointment (given 10 g/kg)

Ointments formulated with the leaves of *Smilax china linn*., aqueous extract were evaluated for their wound healing activity on Male *Wistar* rats using the excision wound healing model.

Excision wound model

Excision wound model as per the method described was used. Three groups of animals containing six rats in each group were anesthetized by open mask method with anesthetic ether. The rats were depilated on the back and a predetermined area of 500 mm^2 full thickness skins was excised in the dorsal interscapular region. Rats were left undressed to the open environment. The formulation ointment and standard drug were applied daily until the complete healing. In this model, wound contraction was monitored. The wound areas were measured while the animals were under an anesthesia on the 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 day after surgery. The resulting data were analysed statistically using the One-way Analysis of Variance (ANOVA), and the significant means were separated using the Duncan multiple range test[38].

TABLE No - 5

THE EFFECT OF SMILAX CHINA LEAVES AQUEOUS EXTRACT OINTMENT AND LEPAM FORMULATIONS ON EXCISION WOUND HEALING IN RATS.

Group	Dose	Wound area in mm^2						
		Day 1	Day 4	Day 6	Day10	Day 12	Day 14	Day 17
Control	-	225.3±0.12	210.3± 0.09	190.5± 0.17	90.5± 0.09	80.30± 0.10	65.3± 0.11	55.2± 0.09
Standard		334±0.37	281±0.30	222±0.24	152±0.16	96 ± 0.06	19 ± 0.02	9±0.01
Smilax china ointment	500 mg	285±0.38	287±0.31	248±0.25	228±0.15	109±0.08	23 ± 0.03	10 ± 0.02

C means control and **AQ EX** means aqueous extract **p < 0.05

Statistical analysis : The data were analyzed using one way analysis of variance (ANOVA) and data subjected to LSD post hoc test. Differences in mean between paired observations were accepted as significant at P≤0.05.

GRAPH NO : 1

THE EFFECT OF SMILAX CHINA., LEAVES AQUEOUS EXTRACT OINTMENT ON EXCISION WOUND HEALING IN RATS.

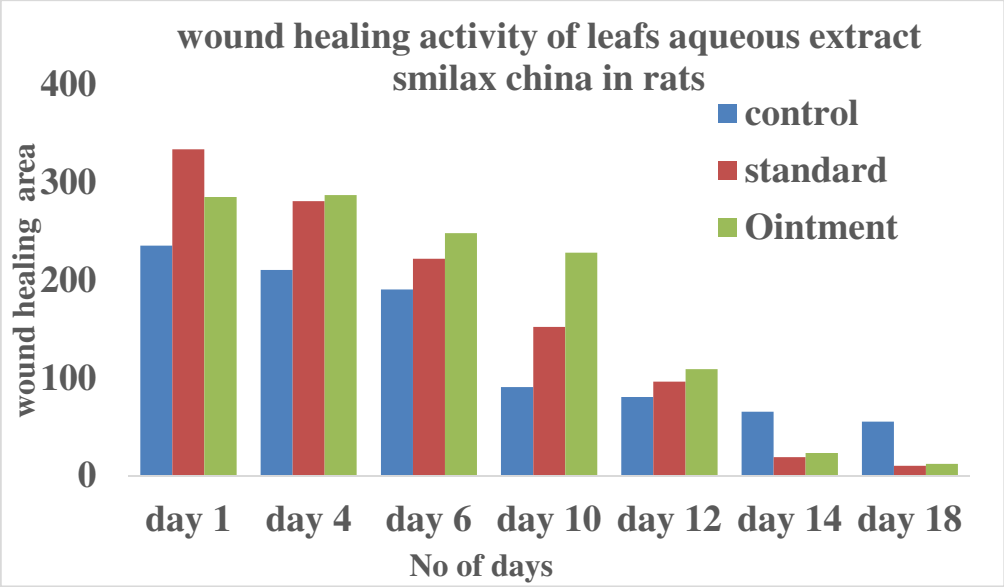
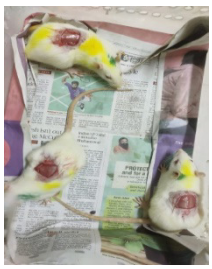


Figure . 1

Photographs of rats showing various phases of wound healing.

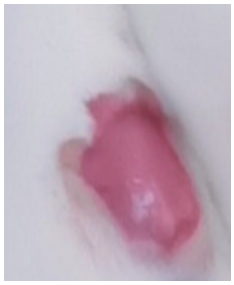
A: Control;



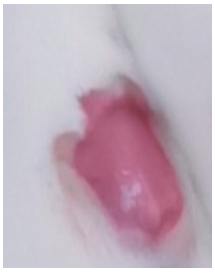
DAY- 1

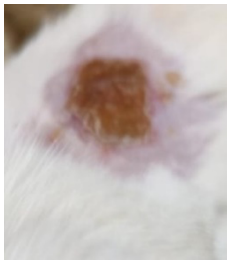
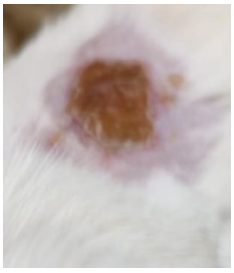
DAY- 4

DAY- 6



DAY-10

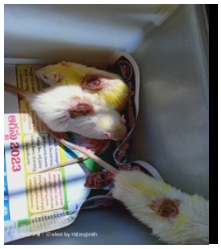


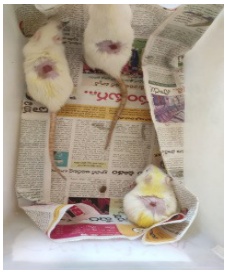
DAY-12 **DAY-14** **DAY-17** **DAY-18**

B: smilax china., leaves aqueous extract ointment 500mg/kg;



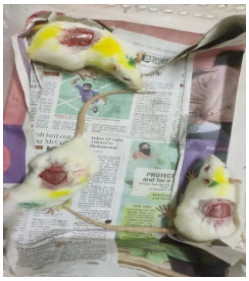
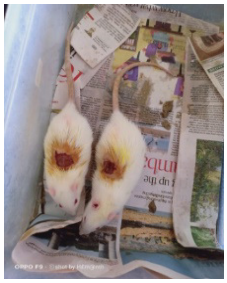



DAY-1 **DAY-4** **DAY- 6** **DAY- 10**

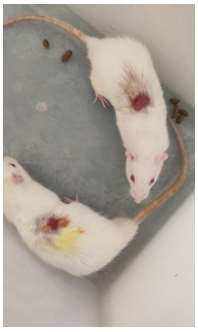



DAY- 12 **DAY- 14** **DAY- 17** **DAY-18**

C: povidone iodine 5%w/w ointment.

DAY - 1 **DAY - 4** **DAY - 6** **DAY - 10**

DAY - 12 **DAY - 14** **DAY - 17** **DAY - 18**

A: Control; B: *smilax china.,* leaves aqueous extract ointment 500mg/kg; C: povidone iodine 5%w/w ointment.

RESULTS AND DISCUSSION

The aqueous extracts were subjected to Phytochemical test to find out the active constituents which shows the presence of alkaloids, flavanoids, glycosides, saponins and tannins results are shown in Tables:1.

Physical evaluation of ointments

Organoleptic characteristics

The organoleptic properties, including physical appearance, color, texture, phase separation, homogeneity, and immediate skin feel of the ointment formulations, are shown in Table 3. Results showed that the ointments had a good appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation. All formulations were white in color and aromatic odor.

pH

pH of all formulations was found to be between 6.80±0.152 and 7.02±0.174 that is within the range, which are presented in Table 4. The pH of all formulations lies in the normal pH range of the skin. Viscosity of all the formulations was noted and found in the range of 2314±6.13–2851±9.93 CPS at 10 rpm as shown in Table 9. All the formulations were showed pseudoplastic flow. Average of three readings was calculated and standard deviation was determined (n=3).

Spreadability Ointment

spreadability can be categorized into three groups: Low, moderate, and high. After screening, it was found to be inversely proportional to the concentration of

sunflower. As the amount of sunflower wax increased, the ointment became thicker, and, consequently, spreadability decreased. The spreadability of all formulations was determined and it was observed that formulation F2 has greater spreadability as compared to other formulations as shown in Table 4.

Hardness

Hardness test is indicative of strength of ointment formulations and the results are found in the range of 122±5.03–243±4.13 g. It is observed that hardness of the ointment base formulated increases with increase in sunflower wax. This indicates that the proportion of the sunflower wax must be well controlled in the formulation for optimal hardness because when the product is too hard, spreadability will be difficult, and thus, the efficacy will be retarded. Optimized formulation F2 showed closed strength represented in Table 4.

In vitro drug diffusion study

The in vitro release profile using salicylic acid as model drug from ointment diffusion cell showed that 94.34% of drug was released at the end of 1 h. Ex vivo permeation study The results of drug permeation through rat skin reveal that drug was released continuously through rat skin over a period of 1 h. The F2 formulation with 500mg smilax china linn., wax showed drug permeation of 82.57±1.25 .

Skin irritation study

Optimized ointment formulation did not show any sign of erythema or edema when applied topically to the skin of animals during the study period. Formulation F2 formulations were found to be safe as they do not cause redness of skin and get score 0.

Stability study

All the ointment formulations were subjected to stability study as per ICH guidelines and results are shown in Table 4. During the stability studies, the appearance of formulations was clear and no significant variation in pH, spreadability, viscosity, and drug content for optimized formulation for the period of 3 months.

In order to add good cosmetic value to the powder, it was made of fine quality. The results of the evaluation parameters have been mentioned in table 4. Characterization of the formulation was done for its organoleptic, physiochemical properties, and skin irritation. The formulation has been tested for its colour, odor, touch, consistency, texture, spread ability and moisture content. Additionally, the skin irritation test was also performed for the formulation. Based on the analysis of pH and Spread ability, it was found that the pH of the ointment formulation was 7.01 ± 01.85, and the spreadability was 109.08. Having a greasy texture, characteristic odor, soft consistency, and gritty texture, the formulation is lightweight.

It was found that the formulation was easy to apply, easy to wash, and slightly alkaline pH compatible with normal skin physiology. There were no signs of skin dryness. The formulation has moisturizing constituents, which offers no need for extra moisturizing agents. Based on the results, the formulation proved suitable for the application, comply with the requirements and do not cause irritation.

Pharmacological studies:

The acute oral toxicity of aqueous extract of the leaves of *Smilax china linn.,* was carried out as per OECD-420 guide lines. The acute toxicity studies revealed that LD_{50} > 800 mg for aqueous extract. Therefore we selected 300mg/kg, 500mg/kg and 1000mg/kg of aqueous extract of leaves of *Smilax china linn.,* .

Aqueous extract of leaves of *Smilax china linn.,* was studied for its therapeutically effective role in excision model of wound healing activity by formulated ointment .

WOUND HEALING ACTIVITY

In this study, topical application of the Aqueous extract of leaves of *Smilax china Linn*., incorporated into an ointment base on the excision wound in rats caused a significantly ($P<0.05$) higher rate of wound healing. Application of the ointment batch containing the concentrations of leaves of *Smilax chinalinn.,* extract (500 mg/kg, in 100g of ointment, 600 mg/kg, in 10g of ointment) produced the highest rate of wound healing, reducing the wound healing area to 15 days compared to the

standard ointment treatment with wound healing area of 18 days (Table No - 5). Wound healing is a natural process of regenerating dermal and epidermal tissues. Whenever there is a wound, a set of overlapping events takes place to repair the damage. On comparison of two formulations of tested ointment leaves of *Smilax china linn.,* extract of 500 mg/kg showed good contraction of wounds as shown in fig - 1 .

The result of this work shows that formulating leaves of *Smilax china linn*., extracts into an ointment is effective in wound repairs as shown in Graph No - 1 and encourages the harnessing of the extracts in the formulation of commercial dermatological ointments.

Conclusion:

The leaves of *Smilax china linn.,* has been examined to gain an insight of its Pharmacognostical, Preliminary Phytochemical and pharmacological studies.

The phytochemical investigation showed the presence of alkaloids, flavanoids, glycosides, fixed oils, saponins, proteins and tannins were identified.

It is seen that ointment containing a *Smilax china linn.,* with active ingredient showed good strength, viscosity, and spreadability with no signs of skin irritation on rat skin. This work will encourage more research and faith toward utilization of natural active ingredients in pharmaceuticals. There is a drastic rise in demand for herbal formulations because the belief that they are safer and cause fewer side effects than synthetic formulations. A newly developed ointment product has exhibited good wound healing activity properties.

The pharmacological studies showed significant ointment on excision model of wound healing activity properties at the dose of 500mg/kg with aqueous extracts. When compared ointment formulations F2 formulation ointment showing more therapeutic activity than F1 and F3 in wound healing activity and, ointment F2 showing maximum good therapeutic activity When compared to standard .

Therefore studies are needed to isolate and characterize the active principle of leaves of Smilax china., which can have offer wound healing activity properties and to establish its mechanism of action.

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1. Vipin Kumar Garg et al., (2011) reported the Wound-healing activity of ethanolic and aqueous extracts of *Ficus benghalensis,* J Adv Pharm Technol Res. 2011 Apr-Jun; 2(2): 110–114.
2. Raina R, Prawez S, Verma PK, Pankaj NK. Medicinal plants and their role in wound healing. *VetScan.* 2008;3:1–7.
3. Okwu, D E(2001): Evaluation of the chemical composition of indigenous spices and flavoring agents, Global .J.Pure and applied sciences 7(3): 455-459
4. Hill, A F (1952) Economic Botany . A textbook of useful plants and plant products. 2nd edition.McGraw –Hill Book company.Inc. New York.
5. Beebe DJ, Mensing GA, Walker GM. Physics and applications of microfluidics in biology. Annu Rev Biomed Eng 2002;4(1):261–286. [PubMed: 12117759].
6. Ibn Rushd. Kitabul kulliyat. first edi. CCRUM Publication, New Delhi. 1980; (1): 105-10.
7. Benedek TG, Rondnan GP. A brief history of the rheumatic disease. Bull Rheum Dis. 1982; 32:59-68
8. Nuki G, Simkin PA. A concise history of gout and hyperuricemia and their treatment. Arthritis Res Ther. 2006; 8(suppl 1): S1
9. Unwood C.E. General and Systematic Pathology. 3rd edi. Churchill Livingstone; 2000: 726-27, 131-32, 577
10. Brett R et al., (2020) reported the Treatment of Psoriatic Arthritis With Acupuncture, Turmeric (*Curcuma longa*), Sarsaparilla (*Smilax officinalis*) and

Vitamin D. Journal of Chiropractic Medicine Volume 19, Issue 3, September 2020, Pages 194-200

11. Chen Wang et al., (2022) reported the Scopolin obtained from Smilax china L. against hepatocellular carcinoma by inhibiting glycolysis: A network pharmacology and experimental study J Ethnopharmacol. 2022 Oct 5;296:115469. doi: 10.1016/j.jep.2022.115469. Epub 2022 Jun 16.
12. Xin Li et al., (2022) reported the Smilax china L. flavonoid alleviates HFHS-induced inflammation by regulating the gut-liver axis in mice Phytomedicine. 2022 Jan;95:153728. doi: 10.1016/j.phymed.2021.153728. Epub 2021 Sep 5.
13. Si-Yi Jiang et al., (2019) reported the Spectrum-effect relationship between UPLC fingerprint of Smilax china and anti-pelvic inflammation in rats. Zhongguo Zhong Yao Za Zhi. 2019 Aug;44(15):3323-3329. doi: 10.19540/j.cnki.cjcmm.20190523.302.
14. Kyoung Jin Nho et al., (2015) reported the Anti-metastatic effect of Smilax china L. extract on MDA-MB-231 cells Mol Med Rep. 2015 Jan;11(1):499-502. doi: 10.3892/mmr.2014.2698. Epub 2014 Oct 17.
15. Wei-Xin Wang et al., (2013) reported the Tumoral cytotoxic and antioxidative phenylpropanoid glycosides in Smilax riparia A. DC J Ethnopharmacol. 2013 Sep 16;149(2):527-32.doi: 10.1016/j.jep.2013.07.011. Epub 2013 Jul 26.
16. Xu PL et al., (2022) reported the Matrine injection inhibits pancreatic cancer growth via modulating carbonic anhydrases- a network pharmacology-based study with in vitro validation J Ethnopharmacol. 2022 Apr 6;287:114691. doi: 10.1016/j.jep.2021.114691. Epub 2021 Sep 29.
17. Tiantian She et al., (2015) reported the Sarsaparilla (Smilax Glabra Rhizome) extract inhibits migration and invasion of cancer cells by suppressing TGF-β1 pathway PLoS One. 2015 Mar 5;10(3):e0118287. doi: 10.1371/journal.pone.0118287. eCollection 2015.
18. Victor Ruberio Lincha et al., (2016) reported the Effects of Constituent Compounds of Smilax china on Nicotine-Induced Endothelial Dysfunction in

Human Umbilical Vein Endothelial Cells Biol Pharm Bull. 2016;39(6):984-92. doi: 10.1248/bpb.b15-00997.

19. Hee Eun Lee et al., (2017) reported the Chemical Constituents of Smilax china L. Stems and Their Inhibitory Activities against Glycation, Aldose Reductase, α-Glucosidase, and Lipase Molecules. 2017 Mar 11;22(3):451. doi: 10.3390/molecules22030451.
20. Gonzales G.B., Smagghe G., Grootaert C., Zotti M., Raes K., Camp J.V. Flavonoid interactions during digestion, absorption, distribution and metabolism: A sequential structure-activity/property relationship-based approach in the study of bioavailability and bioactivity. *Drug Metab. Rev.* 2015;47:175–190. doi: 10.3109/03602532.2014.1003649. PubMed CrossRef Google Scholar.
21. Vijayalakshmi A, Ravichandiran V, Malarkodi V, Nirmala S, Jayakumari S. Screening of flavonoid "quercetin"from the rhizome of Smilax china Linn. for anti-psoriatic activity. Asian Paci□c Journal of Tropical Medicine 2012;4: 269-27.
22. Lee, S.I.; Lee, Y.K.; Kim, S.D.; Cheng, J.; Yang, S.H.; Suh, J.W. Xanthine and aldehyde oxidase inhibitory activities, and antihyperuricemic effects of fermented Smilax china L. Leaf extracts and fractions. J. Appl.Biol. Chem. 2014, 57, 53–59. [CrossRef]
23. Shao, B.; Guo, H.Z.; Cui, Y.J.; Liu, A.H.; Yu, H.L.; Guo, H.; Xu, M.; Guo, D.A. Simultaneous determination of six major stilbenes and flavonoids in Smilax china by high performance liquid chromatography. J. Pharm.Biomed. Anal. 2007, 44, 737–742. [CrossRef] [PubMed]
24. Jin, T.Y.; Park, J.R.; Kim, J.H. Electron donating abilities, nitrite scavenging effects and antimicrobial activities of Smilax china leaf. J. Korean Soc. Food Sci. Nutr. 2004, 33, 621–625.
25. Kokate C.K.,Pactical Pharmacognosy Vallabh Prakashan, Delhi, 4th edition.2001.,107-111, 123-125,130

26. Evans WC. Trease and Evans Pharmacognosy. 15th edn. W.B Saunders. 2002: 336
27. Indian pharmacopoeia, 1996,Govt. of India, Ministry of Health and Family Welfare, Controller of Publication, Delhi. 75-81, 947-949, A7071, A74,A76,A89,A105,A106.
28. Indian pharmacopoeia, 1996,Govt. of India, Ministry of Health and Family Welfare, Controller of Publication, Delhi. 75-81, 947-949, A7071, A74,A76,A89,A105,A106.
29. Morrison WH. Variation in the wax content of sunflower seeds with location and hybrids. J Am Oil Chem Soc 1983;60:1013-5.
30. Ayerbe R. A study on the recovery of byproducts from the dewaxing of sunflower oils of national production. Grasas Aceites 1985;36:1-4.
31. Wannee J. Review of nutraceutical uses of an antioxidant sunflower sprouts. Asian J Pharm Clin Res 2016;9:21-3.
32. Mariani C. Minor components of vegetable oils: Non-glyceridic esters. Riv Ital Sostanze Grasse 1989;66:397-401.
33. Carelli AA. Wax composition of sunflower seed oils. J Am Oil Chem Soc 2002;79:763-8.
34. Sharma PP. Cosmetics-formulation, Manufacturing and Quality Control, Cap. 7. 5th ed. Delhi, India: Vandana Publications; 2014. p. 181-91.
35. Rowe RC, Sheskey PJ, Weller PJ. Handbook of Pharmaceutical Excipients. London: Pharmaceutical Press; 2003.
36. Bakhari N.A., Sadikun A., Choon, T.S., Ying, T.S and Asmawi, M.Z., "Aporphine Alkaloids Isolated from the Cardiovascular Active Fraction of Tinosporacrispa," Malaysian Journal of Science, vol. 24(1), 2005, pp.161-165.
37. M.R.Sulaiman, Z.A.Zakaria and R.Lihan, "Antinociceptive and Anti-inflammatory Activities of Tinosporacrispa in Various Animal Models," International Journal of Tropical Medicine, vol. 3(3), 2008, pp.66-69.

38. Swati Rawat and Akhilesh Gupta, “Development and Study of Wound Healing Activity of an AyurvedicFormulation,” Asian Journal of Research in Pharmaceutical Sciences, vol. 1(1), 2011, pp.26-28.