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Preparation of Wound Healing Gel and its Evaluation

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Abstract: *In this present study, we have prepared herbal gel for wound management and its batches. currently issues related to the wound and its repairing mechanism time taking process. Required material for the preparation of gel we taken by the common process we have prepared gel. By different method the formulation was prepared and further its evaluation test was performed. Out of this batch last HG were found effective and best as comparative other batches. Henceforth as per our need herbal gel formulation were prepared and its ideal evaltuion process were carried out.*

Keywords*:* Herbal gel, Wound healing, Evaluation

I. INTRODUCTION

In human being, skin is the most susceptible part for entering of various pathogens, microorganisms and spreading of diseases. Prolong use of antibiotics may leads to develop antibiotic resistance and various side effects such as erythema, photosensitivity, dermatitis, excessive skin irritation, urinary problem, joint and muscle pain, headache, depression etc.
Moreover, Indian folk medicine includes numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhea, scabies, venereal disease, ulcers, snake bite, etc. Moreover, these medicinal plants are easily available, cost effective and commonly used by the human beings. Taking these facts into considerations, the present study was designed to systematically explore and analyze the phytoconstituents of selected plant extracts and formulate a new polyherbal gel formulation.India is a hub for medicinal plants, about 15% out of the 20,000 medicinal plants of the world is found growing wild in different climatic conditions. There is a need for continuous search of indigenous drugs which can provide cheaper and better therapeutic efficacy. Literatures have proven that plants of varying potency when combined theoretically might produce the synergistic therapeutic effect. The reason for the synergistic effect of the polyherbal formulation might be due to the potentiating effects of other plants active constituent's leads to require lower dose to produce the desired therapeutic effect which can improve patient's compliance.
The growing popularity of natural and herbal medications, easy availability of raw materials, cost- effectiveness and paucity of reported adverse reaction, prompted us formulate a polyherbal topical preparation and assess its wound healing ability. The combination is used in order to enhance the wound healing activity

II. OBJECTIVES

The objectives of this study –

1. Formulation of Polyherbal gel.
2. To study the wound healing activity of prepared gel using invitroassay.

III. REVIEW OF LITERATURE

The literature survey carried out on the proposed topic by referring various scientific journals, book website on internet the survey reveals that no such articles were reported on the proposed work and some related articles are mentioned below
Shifali Thakur et al, It is recommended by the World health organization (WHO) that most of the world's population depends on herbal medicine for their health care. Nigella sativa is commonly known as a Black seed, Black cumin or

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'Habbatul Barakah' i.e. an annual herb possessing a wide range of medicinal uses apart from its commercial significance as a spice yielding plant. It has long been used in the folk medicine system of the Arabian Gulf region, Far East Asia, and Europe. Seeds and oils of N. Sativa are the primary medicinal source from ancient times. It is one of the most common herbal plants used worldwide and possesses various chemical constituents such as thymoquinone, thymohydroquinone, dithymoquinone, thymol, nigellicine, carvacrol, nigellicine, nigllimine, nigellidine, and alpha-hederin. There are more than 100 constituents isolated from the plant. These chemical compounds have many pharmacological activities such as antibacterial, antiviral, anti-inflammatory, wound healing effect and also for acne vulgaris, skin cancer, pigmentation and many other cosmeceutical properties. Much scientific research on N. sativa has been conducted over the last five decades to investigate chemical and pharmacological properties. In this review, the phytochemicals, pharmacological properties, Ayurvedic properties and folk uses of Nigella sativa are briefly explained.

Alireza Tavakkoli et al,Nigella sativa (black seed or black cumin), which belongs to the Ranunculacea family, is an annual herb with many pharmacological properties. Among its many active constituents, thymoquinone (TQ) is the most abundant constituent of the volatile oil of Nigella sativa (N. sativa) seeds, and it is the constituent to which most properties of this herb are attributed. Methods: PubMed-Medline, Scopus, and Web of Science databases were searched to identify randomized control trials (RCTs) investigating the therapeutic effects of N. sativa and/or TQ. In this review, we investigated the clinical uses of N. sativa and TQ in the prevention and the treatment of different diseases and morbidity conditions in humans. Black seed and TQ are shown to possess multiple useful effects for the treatment of patients with several diseases, such as inflammatory and auto-immune disorders, as well as metabolic syndrome. Also, other advantages, including antimicrobial, anti-nociceptive and anti-epileptic properties, have been documented. The side effects of this herbal medicine appear not to be serious, so it can be applied in clinical trials because of its many advantages.

Samra Ashfaq et al,Nigella sativa originated from the Mediterranean region. It belongs to the family Ranunculaceae (butter-cup family). Used as spice and also generally used therapeutic plant all over the world. Customarily it is used to treat allergic disorders. Important medication in the Indian conventional methods of medication like Ayurveda (herbs) and Unani (roots of plants). Seeds are used as flavoring agent and preservatives. Black cumin seeds were extensively utilized for the cure of various ailments and conditions. In Islamic Literature, it is recognized as one of the ultimate forms of therapeutic medication. It has been widely used as aromatic, respiratory stimulant, diuretic, hypoglycemic, antitumor and an analgesic. N. sativa oil also have therapeutic properties and many active compounds. It is usually extracted by hydro-distillation method. Thymoquinone isolated from the black cumin is the principle main dynamic constituent of its volatile oil that involves in numerous biochemical and physical properties of this plant. The current review paper tries to describe the essential oils of N. sativa, their chemical composition as well as therapeutic activities.

Md. Abdul Hannan et al,Mounting evidence support the potential benefits of functional foods or nutraceuticals for human health and diseases. Black cumin (Nigella sativa L.), a highly valued nutraceutical herb with a wide array of health benefits, has attracted growing interest from health- conscious individuals, the scientific community, and pharmaceutical industries. The pleiotropic pharmacological effects of black cumin, and its main bioactive component thymoquinone (TQ), have been manifested by their ability to attenuate oxidative stress and inflammation, and to promote immunity, cell survival, and energy metabolism, which underlie diverse health benefits, including protection against metabolic, cardiovascular, digestive, hepatic, renal, respiratory, reproductive, and neurological disorders, cancerand so on. Furthermore, black cumin acts as an antidote, mitigating various toxicities and druginduced side effects. Despite significant advances in pharmacological benefits, this miracle herb and its active components are still far from their clinical application. This review begins with highlighting the research trends in black cumin and revisiting phytochemical profiles. Subsequently, pharmacological attributes and health benefits of black cumin and TQ are critically reviewed. We overview molecular pharmacology to gain insight into the underlying mechanism of health benefits. Issues related to pharmacokinetic herb–drug interactions, drug delivery, and safety are also addressed. Identifying knowledge gaps, our current effort will direct future research to advance potential applications of black cumin and TQ in health and diseases.

HalaGali-Muhtasib, et al, The seeds of Nigella sativa L., commonly known as black seed, have been used in traditional medicine by many Asian, Middle Eastern and Far Eastern Countries to treat headache, coughs, abdominal pain, diarrhea, asthma, rheumatism and other diseases. The seeds of this plant are the most extensively studied, both

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phytochemically and pharmacologically. The aqueous and oil extracts of the seeds have been shown to possess antioxidant, antiinflammatory, anticancer, analgesic and antimicrobial activities. Thymoquinone, the most abundant constituent of black seed essential oil, has been shown to be the active principle responsible for many of the seed's beneficial effects. This review paper describes the seed, its chemical components and popular uses in traditional medicine. The paper also discusses the medicinal potential and therapeutic values of some of the individual components present in the extracts of the seeds.

Elena A. Stepanycheva et al.This study is devoted to an estimation of the action of preparations based on Pongamia pinnata oil on the life cycle (survival, fecundity) of green peach aphid Myzuspersicae (Sulzer) (Hemiptera: Aphididae). The M. persicae is a widespread pest and damages more than 100 species of plants. All test formulations had aphicidal activity for M. persicae adults and larvae. Moreover, they possess prolonged action, exerting a negative influence on the offspring. The preparations differed in speed of onset of mortality. The single treatment with these formulations provides significant reduction in the number of aphids during the observation period, because of the efficiency rising in time.

AlokePurkait et al, Pongamia pinnata (L.) seed oil is effective for its insecticidal and larvicidal activities. However, its low aqueous solubility, high photosensitivity, and high volatility restrict its application for the control of agricultural pests. Encapsulation can be an effective technique to overcome such hindrances. Therefore, P. pinnata oil (PO) was extracted from its seeds and analyzed for karanjin content (3.18%) by GC-MS/MS as the marker compound. Microencapsulation (MC) of PO was prepared by interfacial polymerization between isocyanates and polyamine and tested for insecticidal and larvicidal activities. Bioassay of the developed formulations was tested in-vitro against 2nd instar larvae of Bombyx mori (Bivoltine hybrid) and in-vivo insecticidal bio-efficacy was tested against aubergine aphid (Aphis gossypii G.) and whitefly (Bemisiatabaci G.). Various properties of micro-capsules viz., stability, size, oil content and release kinetics were examined. Average diameter of capsules (1 μm) with Zeta potential (-16 mV) was indicated by the Dynamic Light Scattering (DLS) instrument. Existence of PO in the microcapsules was confirmed by an optical microscopic study. Spectroscopic analysis revealed 87.4% of PO was encapsulated in polyurea shell. The shelf-life (T10), half-life (T50), and expiry- life (T90) of polyurea coated capsules were 11.4, 75.3 and 250.0 h, respectively. Polyurea coated PO capsule formulation showed evidence of in-vitro toxicity against 2nd instar larvae of B. mori (LC50 ¼ 1.1%; LC90 ¼ 5.9%). The PO formulation also exhibited 67.0–71.8% and 62.4–74.8% control of aphid and whitefly population in aubergine at 4.0% dose following 7–14 days after application. The study unveiled its significance in developing controlled release herbal formulations of P. pinnata as an alternative to harmful conventional synthetic insecticides for crop protection.

Vigya Kesari et al,Oil analysis and antimicrobial activity from seeds of elite genotype of Pongamia pinnata was carried out in the current study. The highest oil yield (33%) from seeds was recovered in n-Hexane. Physico-chemical properties of crude oil established suitability of P. pinnata for its use as a potential biofuel crop. The total mono unsaturated fatty acid (oleic acid 46%) present in seed oil was more in comparison to polyunsaturated fatty acid (33%) as analyzed by GC–MS. Seed oil also showed inhibition against the tested fungal and bacterial cultures. However, the efficacy of antimicrobial activity of the seed oil at four concentration levels (50%, 80%, 90% and 100%) against various pathogenic indicators was found to be concentration- dependent. The obtained results confirmed the use of seed oil from well characterized elite genotype of Pongamia as diesel fuel and in pharmaceuticals.

Rahul Deo Yadav et al,The word herb, as used in herbal medicine, is also known as botanical medicine or as Phytotherapy or Phytomedicine which means a plant or plant part is used to make medicine to assist in the healing process during illness and disease. So there are many herbal remedies individually or in combination have been recommended in various medicinal treatises for the cure of different diseases. It contains various phytoconstituents belonging to alkaloids, glycosides, flavonoids, fixed oils, and carbohydrates. The roots of Pongamia pinnata are good for cleaning foul ulcers, cleaning teeth, strengthening gums and gonorrhoea. The root paste is used for local application in scrofulous enlargement. The fresh bark of Pongamia pinnata is sweet and mucilaginous to taste, soon become bitter and acrid. It is antihelmintic and useful in beri-beri, ophthalmology, dermatopathy, vaginopathy, and ulcers. Leaves of Pongamia pinnata are digestive, laxative, antihelmintic and are good for diarrhea, leprosy, dyspepsia and cough. Flowers are useful to quench dipsia in diabetes and for alleviating vata and kapha. The seeds are antihelmintic, bitter, acrid, haematinic and carminative. They are useful in inflammation, chronic fevers, anaemia and hemorrhoids. The oil

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is antihelmintic, styptic and recommended for opthalmia, leprosy, ulcers, herpes and lumbago. Its oil is a source of biodiesel.

L C Meher et al,The present study deals with the transesterification of Pongamia pinnata oil by means of methanol to study the feasibility of methanolysis process by using potassium hydroxide catalysts. The yield of biodiesel obtained was >97 per cent by using oil/methanol molar ratio 12:1, potassium hydroxide as catalyst, at 65oC and stirring at 360 rpm in 3 h. The biodiesel was characterized by TLC and HPLC analysis to determine the fatty acid methyl esters, mono-, di- and triglycerides and glycerol. The properties like viscosity, flash point, cloud point, and pour point have been determined for accessing the fuel quality of karanja based biodiesel.

Gouri Dixit et al,In the present study, three medicinal plants Cynodondactylon (L.) Pers, Cassia tora Linn. and Cassia alata Linn having significant antiinflammatory potential were selected to be formulated as polyherbal gels. The gels were prepared using the dried methanolic extract of Cassia tora Linn, Cassia alata Linn and Cynodondactylon (L.) Pers. Polyherbal gel formulations were evaluated for its pH, appearance and homogeneity, viscosity, spreadability and skin irritation studies. Assessment of Antiinflammatory activity was done by carrageenan induced rat paw edema and formalin- induced rat paw edema. Individual and polyherbal gel of Cassia alataLinn,Cassiatora Linn. and Cynodondactylon (L.) Pers were found to possess anti-inflammatory effect in acute and chronic models. Polyherbal gel also showed synergistic effect as compared to individual gels which can be useful for the treatment of local inflammation.

Sudipta Das et al,The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing Clerodendroninfortunatum extract. The gel formulation was designed by using aqueous extract of Clerodendroninfortunatum leaves in varied concentrations (2.5% and 5%) and evaluated using physiological measurements. The gel was prepared by using various polymer bases (Sodium CMC, Carbopol 934). Among them Carbopol 934 has given better gel formation. The gel was prepared by using Carbopol 934, Clerodendroninfortunatum leaves extract, Propylene glycol 400, Methyl paraban, Propyl paraben and required amount of distilled water. Then skin pH (6.8-7) was maintained by drop wise addition of tri-ethanolamine. The physiochemical parameters of formulations (pH, viscosity, spreadability etc.) were determined. Stability studies have carried out as per ICH guidelines for 3 months at different temperatures and humidity. The results showed that formulation containing 2.5% Clerodendroninfortunatum extract have better stability than other formulation. Further all formulations have studied for skin irritation on animal model (Rabbit) and result showed that there was no skin irritation to animals.

Mohammed Haneefa et al,To formulate Pothos scandens Linn (P. scandens) leaf extract in to a gel and investigate their burn wound healing activity. Ethanolic extract of dried leaves of P. scandens were subjected to priliminery phytochemical evaluation and wound healing activities studies. Different gel formulations of ethanolic extract of P. scandens (4% w/v) were prepared using polymers carbopol 934 and carbopol 940 by varying their concentration. These formulations were evaluated for physical parameters, drug content, pH, viscosity, extrudability, spreadability, primary skin irritation, pharmacological activity and stability. Wound healing studies of ethanolic extract revealed that P. scandens treated animals were found to epithelise in 22 days while the solvent control and the untreated rats epithelise within 35 and 40 days respectively. The formulation with 1.5% w/w carbopol 934 was found to be more promising as it shows better physicochemical characteristics, higher pharmacological activity and stability compared to other formulations. P. scandens alcoholic extract shows significant improvement in burn wound contraction and hence this is a promising candidate in burn wound healing.

Rajurkar B.M et al., reported the ethanol extracts of leaves of Clerodendrum infortunatum Linn, Simarouba glauca and Psoraleacorylifolia possess antimicrobial activity. All ethanolic extracts exhibited significant anti-microbial activity comparable to the standard drug tetracycline. Ethanolic extract of Clerodendrum infortunatum shows inhibitory zone as compared to ethanolic extracts Simarouba glauca and Psoraleacorylifolia. They concluded that some of the components from the mixture of all three extracts exhibit the synergistic action. The isolation of the active component from these extracts that exhibit synergistic action against bacteria.

KhalingMikawlrawng et al., reported as the methanolic and ethanolic extracts of both fresh and dried leaves of Simarouba glauca were tested for their inhibitory activity against pathogenic fungi. Screening of the crude extracts for the antifungal activity using well diffusion assay showed strong inhibition against the tested fungus. Ethanolic extracts of both the fresh and dried leaves were found to be more effective as compared to methanolic extracts against the

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growth of the fungi. That study also showed that the leaf extracts of Simarouba glauca is more effective against Aspergillusparasitic us as compared to Fusariumoxysporum. The antifungal assay showed that Simarouba glauca has antifungal property against the tested fungi Fusariumoxysporum and Aspergillusparasiticus.

T.G Umesh et al., reported as the methanol, ethanol and water extracts of Simarouba glauca leaves were examined for total phenolics, flavonoid and tannin content. Its antioxidant properties using FRAP, Phosphomolybdenum, Ferric ferrozine assays and free radical scavenging using DPPH were determined. Further, the reducing power and iron chelating effect of the extract using spectrophotometric assays were estimated. Finally, the cytotoxic activity against few human cancer cell lines were also examined using MTT assay. The phytochemical investigations revealed that a Simarouba glauca leaf has only 0.14 to 0.18% of flavonoids, 250-400 µg/mg phenolics and 67-200 µg/mg tannin content in various solvent extracts. The extracts exhibited good reducing power with similar EC50 values approximately in the range of 57-61 µg/ml. Further, the leaf extracts showed iron chelation effect which was more pronounced in aqueous extract with IC50 value of 332µg/ml and exhibited very, strong DPPH radical scavenging action with IC50 values ranging from 9-13 µg/ml in various extracts. The methanolic extracts showed good antioxidant potential using FRAP and phopshomolybdenum methods. The methanolic extract showed strong cytotoxic effect on SCC9 cancer cell line and less potent on HCT116 cancer cells.

R.C Baratakkar et al., developed male specific DNA based marker in Simarouba glauca. A total of 50 random decamer primers were used for screening of specific Random Amplified Polymorphic DNA (RAPD) markers in male and female populations. Pair of Sequence Characterized Amplified Region (SCAR) primers designed based on RAPD sequence, amplified a single 1110 base pairs DNA band only in male populations. These SCAR primers may be efficiently used as effective, convenient and reliable molecular markers for sex identification in Simarouba glauca at pre- flowering stages. This would pave the way to screen male and female seedlings for the mass cultivation which in turn save time and economic resources

Ayme Fernandez et al., reported in the present study, an extensive in vitro antimicrobial profiling was performed for three medicinal plants grown in Cuba, namely Simarouba glauca, Melaleucaleucadendron and Artemisia absinthium. Ethanol extracts were tested for their antiprotozoal potential against Trypanosoma b. brucei,Trypanosomacruzi, Leishmaniainfantum and Plasmodium falciparum. Antifungal activities were evaluated against Microsporumcanis and Candida albicans whereas Escherichia coli and Staphylococcus aureus were used as test organisms for antibacterial activity. Cytotoxicity was assessed against human MRC-5 cells. Only

M. leucadendron extract showed selective activity against microorganisms tested. Although Simarouba glauca exhibited strong activity against all protozoa.

Shankara Sharma et al., reported as the acute oral toxicity and antiulcer profile of the chloroform extract of Simarouba glauca extract in albino rats. CSG at the doses of 200 and 400 mg/kg body weight orally was administered to evaluate antiulcer activity by using Ethanol and Indomethacin induced gastric ulcer models in albino rats. Chloroform extract of Simarouba glauca dose dependent inhibition in ethanol induced gastric lesions, causing 82.63% protection at 400 mg/kg and 53.48% protection at 200 mg/kg. CSG dose dependent inhibition in indomethacin induced gastric lesions, causing 62.65% protection at 400 mg/kg and 54.86% protection at 200 mg/kg. All result are found to be statistically significant ($p<0.05$).Results are evaluated as the chloroform extract of the leaves of Simarouba glauca was able to decrease the acidity.

Mali V.V et al, reported as the effect of fluoride concentration (5ppm, 10ppm, 25ppm, 50ppm and 100ppm) on the photosynthetic pigments (chlorophylls and carotenoid content). It was noticed that the total chlorophylls and carotenoids were increased in response to fluoride stress. It indicate that the Simarouba glauca plant tolerate the fluoride stress by improving photosynthetic efficiency.

SnehaKochath Santhosh et al., reported as crude petroleum ether and ethyl acetate extracts from dried leaves of Simarouba glauca were tested for the antibacterial and antioxidant activity. Antibacterial activity was studied using disc diffusion assay against the organisms like Staphylococcus, Salmonella, Bacillus, Klebsiella, Pseudomonas species and Escherichia coli. Antioxidant activity was evaluated using total antioxidant assay, Hydroxyl radical scavenging assay and 2, 2, - diphenyl - 1 - picrylhydrazyl (DPPH) scavenging activity. Both extracts showed significant antioxidant activity in a dose dependent manner. However, ethyl acetate extract was found to be more effective as compared to petroleum ether extract.

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Ramesh HA et al, Urine is used traditionally in India for the treatment of burns and wounds. It is believed that applying urine over the wound increases healing. The present study was carried out to evaluate the effect of human urine, urea (2.5%), and urea (5%) on experimentally induced wounds in rats and compare the effects observed with an antiseptic agent, povidine iodine solution. The models selected were excision wound, incision wound, burn wound and dead space wound. In the excision wound and burn wound models, a significant decrease in period of epithelization and wound contraction-50% was observed in all the treatment groups when compared to control except low dose of urea (2.5%), which showed reduction in only period of epithelization. In the incision wound model, a significant increase in the breaking strength was observed. Human urine treatment orally produced a significant increase in the breaking strength, dry tissue weight and hydroxyproline content in dead space wound model. It was concluded that human urine applied topically or administered orally (10 ml/kg, p.o) possesses wound

M. Karami et al,The wounds are still the health tribulations at the present time. In the present research the effect of a new herbal ointment (Oppyheal) in treatment of rat's wound has been investigated. The effectiveness of the ointment was compared with the Fibrinolysin/DNAse. In this study fifty four male Wistar rats were used. The wound was created on the back cervical skin ofthe animals under anesthesia in three different sizes. The animals in each size group were randomly divided into three groups. The control group did not receive the ointments. The reference group wasgiven the Fibrinolysin/DNAse and the third group was treated with the Oppyheal. The products weretopically used once per day until the wounds of one group were completely healed. The size of thewound area was measured in days 0-20 by a standard reference ruler. The reduction in size of thewound was calculated and analyzed. The recovered skin of all animals were examined histologically,$p<0.05$ was considered as significant. The results showed a significant difference in woundcontraction between the treated groups and the control group ($p<0.0001$). The new skin of ointmenttreated rats showed healing features in comparison to the control group.This study may introduce a suitable topical ointment, Oppyheal, for wound care.

Kuntal Das et al, The present study was carried out to evaluate the wound healing potentialof crude aqueous extract of Stevia rebaudiana (Bertoni) Bertoni, Asteraceae, inexperimental animals. All experiments were conducted following standard procedures.The crude extract was administered topically in graded doses of 150, 250 and 500 mg/kg b.w. was used for evaluating the wound healing potential in excision wound modelfor fourteen days and orally in the incision wound model for ten days, respectively.Povidone iodine ointment was used as standard (5.0% w/w). Dose dependent activitiesresulted in both the wound models when compared to the standard (povidone iodine)and the control. Topical application of crude aqueous extract of S. rebaudiana (500 mg/kg b.w) in excision wound model decreased signifi cantly the wound area by 15th day,i.e. 48.2±2.0 compared with control 94.1±1.2. Epithelization time was decreased from17.3±0.21 to 12.0±0.10 and hydroxylproline content was increased from 32.2±0.11 to67.6±0.10 when compared with control. In incision wound model breaking strength ofwounds, wet and dry granulation of the tissue weight and hydroxyproline were increasedsignificantly from control with AESR. In conclusion, AESR leaves accelerated woundhealing activity in mice and thus supports its traditional use.

Karodi R et al.India has a rich tradition of plant-based knowledge on healthcare. A large number of plants are used by folklore traditions in India for treatment of cuts, wounds and burns. Rubia cordifolia Linn. (Rubiaceae) is popular all over the world for its medicinal uses in skin diseases like eczema, dermatitis, skin ulcers, etc. In India, it is used traditionally for various types of skin diseases. Hence, the present study was aimed to evaluate its scientific validity. The alcoholic extract and the hydrogel of same were investigated for the evaluation of its healing efficiency on excisionwound model in mice. A different formulation of alcoholic extract was topically applied on the excision wound surfaceas a single dose. Wound area and histopathology were used to evaluate the effect on wound healing. The effectproduced by gel, in terms of wound contracting ability, wound closure, decrease in surface area of wound, tissueregeneration at the wound site and histopathological characteristics were significant ($p < 0.01$) in treated mice. Thepresent study thus provides a scientific rationale for the traditional use of this plant in the management of the wounds.

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IV. METHODOLOGY

COLLECTION OF PLANT MATERIAL

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PREPARATION AND COLLECTION OF PLANT MATERIAL

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COLLECTION OF REQUIRED CHEMICALS FOR THE STUDY

↓

EVALUATION OF IN VITRO ACTIVITY BY SCRATCH ASSAY

↓

DATA ANALYSIS AND WRITING RESULTS

↓

WRITING DISCUSSION AND CONCLUSION

V. PLAN OF WORK

Sr. No.	Particulars of the work
1	**Review of Literature.**
2	**Selection and Collection of plant material.**
3	**Selection of probable chemicals required for the project work.**
4	**Preparation of Herbal Gel and its evaluation.**
5	**Evaluation of Gels**
6	**Result and Discussion**
7	**Conclusion.**

Review of Literature: 60% of the literature review covered,75 to 86 reviews, and research articles were referred for work. National and international journals are preferred.

Selection and Collection of Plant Material: Plant material was selected and collected.

Selection of probable chemicals required for the project work: For extraction and isolation of selected plants were done. Neededchemicalswere collected from store department of the college.

Preparation of Herbal Gel.

Procedure: Required oil was taken and chemicals were mixed in proportion. The herbalgel was prepared, and out of this formulation batch,HG5was good and effective.

Table.1 Different herbal gel preparation batches

Sr. No	Composition of Gel in % W/W	Batch HG 1	Batch HG2	Batch HG3	Batch HG4	Batch HG5
1	**Pongamia Pinnata seed oil in ml**	1.3 ml	1.3 ml	1.3 ml	1.3 ml	**1.3ml**
2	**Black cumin seeds oil in ml**	2.5 ml	2.5 ml	2.5 ml	2.5ml	**2.5ml**
3	**Kalonji Oil in ml**	2.5 ml	2.5ml	2.5ml	2.5ml	**2.5ml**
4	**Propylene glycol**	5	5	5	5	**5**
5	**Methyl paraben**	0.2	0.2	0.2	0.2	**0.2**
6	**Carbapol 934**	0.5	1	1.25	1.50	**2**
7	**Dist. water**	100	100	100	100	**100**
8	**Triethanolamine**	Q. S	Q. S	Q. S	Q. S	**Q. S**

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Figure.1 Pongamia Pinnata Seed Oil.

Figure.2 Black Seed Oil.

Figure.3 Kalonji Seed Oil.

Evaluation of Gel:

Table 2. Different evaluation parameters of the batches

Formulation	Appearance	Spreadability	pH
Control formulation	White and Clear	15.7	7.0
Batch HG1	White and Clear	20.56	6.0
Batch HG2	White and Clear	18.45	6.91
Batch HG3	White and Clear	19.35	6.45
Batch HG4	White and Clear	20.1	6.96
Batch HG5	White and Clear	22.1	7.0

Appearance - Colour is important for patient compliance. The prepared gels were inspected visually for clarity, colour and presence of any particle.**(Table 1)**

pH -The pH of gel was determined using digital pH meter. 2 gm herbal gel was stirred in distilled water till a uniform suspension is formed. The volume was made up to 40 ml and pH of the solution was measured.**(Table 1)**

Viscosity-Viscosity of the gel was determined by using (LV) Brookfield viscometer (Dial type). As the system is non-Newtonian spindle no. 4 is used. Viscosity was measured for the fixed time 2 min for 0.3 rpm.

Skin irritation -Ten healthy male and female volunteers were selected for skin irritation testing. 100 mg gel was applied on the area of 2 cm for 6 hours, on the interior surface of the upper arm and covered with cotton bandage. After 6 hrs the sites were cleaned with acetone and readings are made according to the scale given byDraize Criteria. No irritation: 0 Slight irritation: 1 Irritation: 2 **(Table 2)**

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Table.3 Skin Irritation study data for the herbal gel batches.

Treatment	1 Hour	2 Hour	3 Hour	4 Hour	5 Hour	6 Hour
Batch HG1	0	0	0	0	0	0
Batch HG2	0	0	0	0	0	0
Batch HG3	0	0	0	0	0	0
Batch HG4	0	0	0	0	0	0
Batch HG5	0	0	0	0	0	0
Batch HG6	0	0	0	0	0	0

Spreadability–The spreadability of formulations was determined by an apparatus suggested by Multimer45, which was fabricated itself in laboratory and used for slide fixed on wooded block and upper slide with one end tide to glass slide and other end tied with other end tied to weight pan. An excess of gel (2 – 5 gm) was placed in between two glass slides and then 1000 gm weight was placed on slides for 5 min to compress the sample to a uniform thickness. Weight (80 gm) was added to pan.

The time (seconds) required to separate the two slides, was taken as a measure of spreadability. It was calculated using a formula.

$$S = M. L / T$$

Where, S = spreadability

M = weight tied to upper slide

L = length of glass slide T = time is takenin shorter time intervals, to cover the distance of 6.5 cm, indicating better spreadability.

VI. RESULT AND DISCUSSION

The herbal gel was white in color and translucent in appearance and gave smooth feel on application(Table 1,2). PH also maintained throughout the study which was found 6.91 to 7.0. Spreadability was also measured and found to be less variation. The viscosities of developed gels were measured using Brookfield viscometer with spindle and results were found to be good. The gel was non-irritant upon application on to the skin.

VII. CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It is a very good attempt has made to establish the herbal gel containing different oils extract at various concentrations.

The studies revealed that the developed herbal formulations consist of good comparatively better than later other formulation but all the formulations were nonirritant and did not show any skin toxicity.

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