Volume 3, Issue 3, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

PHYTOCHEMICAL ANALYSIS AND WOUND HEALING ACTIVITY OF CALOTROPIS PROCERA LEAVES EXTRACT LOADED WITH EMULGEL IN ANIMAL MODELS

Najm Ud Din[*1], Waqas Ahmad Khan[2], Mariyam Abbas Jaffari[3], Dawood Khan[4], Umer Khitab[5], Sana Tehniat[6], Abdul Samad Khan[7], Zain Akram Rao[8], Zia Ur Rehman[9]

[*1,4,5,6,7,9]*Institute of Biological Sciences Gomal University D I Khan*
[2,8]*Faculty of Pharmacy, Gomal University, Dera Ismail Khan, KP, Pakistan*
[3]*Department of Biological Sciences Government Graduate College Bhakkar*

[*1]najmuddindawar20@gmail.com, [2]waqasahmadkhan011@gmail.com, [3]mariyamabbasjaffri@gmail.com, [4]daud54567@gmail.com, [5]khitabumer839@gmail.com, [6]bingo.birdz@gmail.com, [7]samadyar86@gmail.com, [8]akramzain08@gmail.com, [9]msd 17281@gmail.com

DOI: https://doi.org/10.5281/zenodo.15515658

Keywords

Article History
Received on 16 April 2025
Accepted on 16 May 2025
Published on 26 May 2025

Corresponding Author: *
Najm Ud Din

Abstract

Calotropis procera demonstrates significant pharmacological properties, as evidenced by its antibacterial, antifungal, anti-inflammatory, anti-oxidant and analgesic activities. The ethanolic extract exhibited broad-spectrum antibacterial effects, with the highest zones of inhibition against Staphylococcus aureus (24 mm) and Bacillus subtilis (22 mm). Fractionated extracts (hexane and chloroform) showed dose-dependent activity, with chloroform fractions being more effective. For example, the chloroform fraction at 60 µL/disc achieved a 25 mm zone of inhibition against Staphylococcus aureus. Similarly, the chloroform fraction displayed superior antifungal effects, with a 20 mm inhibition zone against Candida albicans at 60 µg/disc. In-vivo wound healing activity was evaluated on male Sprague Dawley rats weighing 200-250g. The dorsal side of the rats was shaved and properly anaesthesized them with 1.5 mg/kg of Xylazine and 20mg/kg Ketamine and wound created through incision process and 70% ethanol was used to sanitize them 1g dose for a period of 15 days. These results highlight the therapeutic potential of Calotropis procera and support its traditional medicinal applications. Further studies are warranted to isolate and characterize its bioactive compounds for potential drug development.

INTRODUCTION

Health is the primarily priority to save human being from various diseases. For this purpose, search of novel natural resource for medicine to treat disease is the interest of the growing population [1] Though plants have been used for thousands of years for the treatment of health disorders and prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of plant species used throughout the world for the treatment of infectious diseases [2]. The antimicrobial activities of numerous plants have been reported on desease causing microorganisms which are resistant to microbicidal medicine. Chemicals obtained from plants have the power to control microbial growth in wide range of situations

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

and specific case of disease treatment, many studies have the objective to explain the chemical structure of these plant antimicrobials and the methods responsible for the retardation of microbial growth, either isolated or connected with traditional antimicrobials (Silva NCC 2010). Antimicrobial properties of medicinal plant are being increasingly reported from different parts of the world [3]. Over the centuries, traditional plants are used to cure diseases. According to a report of world health organization (W.H.O), 70% of the world population uses medicinal plants to cure diseases through their traditional practitioners. In subcontinent, plant oriented drugs have been used extensively from a very long time. According to a survey conducted by W.H.O, traditional healers treat 65% patients in Srilanka, 60% in Indonesia, 75% in Nepal, 85% in Mayanmer, 80% in India, and 90% in Bangladesh. In Pakistan, 60% of the population, especially in villages is getting health care by traditional practitioners (Hakims), who prescribe herbal preparations [4]. The skin, EmulGel heaviest and largest of human body is the skin. Its main objective is to defend the body's interior from the outside world. Skin is comprised of several cell kindes. In along with act as a barrier of defence, the skin also helps humans keep their bodies at the proper temperature and uses nerve endings to feel their surroundings. Skin is a complex organ. There are more than 1,000 nerve endings, 20 blood arteries, and 650 sweat glands per square inch of skin on average [5]. The skin represents approximately one-seventh of a person's body weight, despite only being a few millimetres thick. With almost 10% of body mass, the skin is the biggest organ in the body and the one that allows for the closest interaction between the body and its surroundings. The skin has four fundamental layers: the stratum-corneum (non-viable epidermal layer), the other layers of the epidermis (viable epidermal layers), the dermis, & the subcutaneous tissues [6]. The wounds are typically divided into four distinct types: incised, acute, open, & chronic. The time of wound recovery might be impacted by a number of factors, including oxygen saturation, age, stress levels, the existence of infections, type of physique, chronic diseases, arterial insufficient supply, and dietary condition. [7]. Healing of wound medications promotes healing of wounds by altering the various stages of repairing damaged tissue. Particular medication's effect may be useful or detrimental based on the mechanism of action, dose, & delivery route used, as well as the precise phase of wound healing. The extracts of plants comprising natural chemicals can improve wound healing as well as decrease creation of scars. Plant-derived bio-active chemicals with anti-bacterial, anti-oxidant, & healing capabilities that can promote blood clotting, combat infection, & speed up the healing process. Numerous investigations have shown that natural compounds can promote wound recovery & act as effective remedies at all stages of the healing procedure. These compounds may serve to improve the process of healing by lowering inflammatory action & creating scars. Plant-based chemicals are thought to be safe, more tolerated, and more affordable than conventional treatments [8]. Extracts from plants & phytochemicals modulate inflamed region by targeting wound-healing tissues, growth regulators, & cytokines. It can promote fibroplasia, angiogenesis, & epithelialization. In simple terms, these organic substances can stimulate the production of fresh arteries, the development of connective tissues, as well as the regrowth of the cells in the skin, these processes are necessary for the healing of wounds. Despite the difficulties in finding and confirming the authenticity of the active particles, currently, still an array of clinical trials on herbal remedies. These investigations have shed light on the possible advantages and efficacy of employing plant extracts as well as phytochemicals for wound [9].

Materials and Methods

Materials Used

Calotropis procera leaves, methanol, distilled water, filter papers, Wagner's reagent, lead acetate, ferric chloride, acetic anhydride, chloroform, sodium hydroxide, HCL and Carbopol as gelling agent.

Equipment's Used

Grinder, weighing balance, shaking incubator, mechanical or magnetic stirrer, rotary evaporator, and homogenizer.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Plant Collection and Identification

Fresh leaves of Calotropis procera were collected from mature trees located in North Waziristan (KPK) Pakistan, during the peak growing season to ensure optimal phytochemical yield. Carefully taken healthy leaves, avoiding those that show signs of disease or pest damage. The collected samples were properly labeled, placed in clean plastic bags, and transported to the laboratory for immediate processing and identification by a taxonomist.

Drying and Grinding

The drying and grinding process was performed as per previous protocols [10]. The leaves were washed thoroughly with water to remove any dirt or contaminants and air-dried in the shade to prevent degradation of sensitive phytochemicals. Once dried, the leaves were ground into a fine powder by means of a mechanical miller. The achieved powdered material was kept in air-tight containers away from light and moisture until extraction.

Preparation of Extract

The extract was prepared with little modification. The plant powder was macerated in methanol in the ratio of 1 gram of leaf powder into 10 mL of methanol for 72 hours. Whatmann filter paper was utilized for filtration and the filtrate was subjected to rotary evaporator for extract production. The prepared extract was collected from rotary evaporator and kept at 4 °C for further characterization [11]. FTIR was carried out for determining the functional groups and also the compatibility study. Calotropis procera plant's leaves extract was subjected for phyto-chemical analysis, in which all the present compound was determined and measure which is present in the prepared extract. Various tests were applied for this study. All the primary and secondary metabolites were determined by applying different assays. In these metabolites' flavonoids, tannins, alkaloids, saponins, glycosides, phenolic compounds were checked [12].

Antioxidant evaluation

Calotropis procera leaves extract has the anti-oxidant capability and having potential for wound healing. DPPH (2,2 diphenyl-1- picrylhydrazyl) technique was established for performing the anti-oxidant activity of leaf extract. The approach of DPPH is an inactive free-radical molecule. This approach was checked with purple color which absorbed 517nm wave length. The DPPH assay purple color change is dependent on free radical scavenging properties which are present in the extract of any medicinal plant. If the purple color changes then its is confirmed that the plant has potential for scavenging activity. Slight modification was carried out in the method of DPPH, which is already discussed in previous researches [13]. 0.1 mM solution of DPPH was used for 1 mL of methanol-based extract. The sample was then incubated (20 minutes). Then absorbance was measured at 513 nm wavelength. As a reference solution the ascorbic-acid of 1mM was utilized to carried out the DPPH activity. After that by equation the calculation was determined (Gulcin & Alwasel, 2023).

$$\text{DPPH \% inhibition} = \frac{AB - AA}{AB} \times 100$$

where,

'AB' represents the absorbance of 'DPPH' radicals + methanol solvent;

'AA' denotes the absorbance of DPPH radicals + samples extract or standard.

3.7. Preparation of Emulgel

The emulgel was study with minor modification. In the first step an oil in water (O/W) emulsion was fabricated by combining aqueous containing 10 % Calotropis procera leaves extract and oil phases. It was stabilized by the addition of a suitable emulsifier/surfactants. In the second step, a gel solution was ready by adding 2 g of gelling agent (Carbopol) into the distilled water. The prepared gel & emulsion were mixed together in 1:1 ratio to form emulgel. Stability of emulgel prepared emulgel was evaluated for its stability according to ICH (International Conference on Hominization) **pH** of emulgel avoid skin irritation, the pH of the emulgel was maintained 4.5-5.5 according to the pH of the skin [14]

In vivo wound healing activity selection of animal and ethical approval

To evaluate wound healing activity of Calotropis procera extract loaded emulgel, rats as test animals were selected. The study was presented to the Ethical

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Review Board (ERB) of Gomal University, D.I. Khan for proper approval of the study.

Induction of wounds

The animals were injected by xylazine (1.5 mg/kg) and ketamine (20 mg/kg) injection to make them unconscious. The dorsal surface of the rats/rabbits was shaved and sanitized with (70 %) ethanol. A surgical blade was used to make a 10 mm incision on the dorsal side of the animals.

Treatment protocols

Three groups were made from animals and each group containing 03 animals (n=3). The first group was control and treated with blank emulgel. The second group was standard group and treated with marketed product. The third group was experimental group, which was treated with Calotropis procera crude extract loaded emulgel formulation. The study was continued until the recovery of wounds in animals.

Statistical analysis

Using SPSS version 20, IBM, one-way Anova and students T test with p value < 0.05 level of significance was used to the data collected.

Results

Characterization of Prepared Extract

The prepared extract was further analyzed via FTIR, phytochemical analysis and antioxidant activities.

4.1.1. FTIR analysis of extract

For the detection as well as confirmation of biochemical groups existence in extract of Calotropis procera was carried out by FTIR, to check functional groups as well as interaction stability of extract when subjected to combined with emulgel formulation. Spectra of Calotropis procera extract is given in figure 4.1. The peak at 3440.28 cm^{-1} show OH stretching alcohol group existence. The peak 2888.48 cm^{-1} and 1455.58 cm^{-1} show the existence of C-H stretch alkanes and C-H bend alkanes, respectively. At 1249.10 cm^{-1} peak alkyl ketone group and at 1349.30 cm^{-1} the C-H rock alkane group is possible to exist.

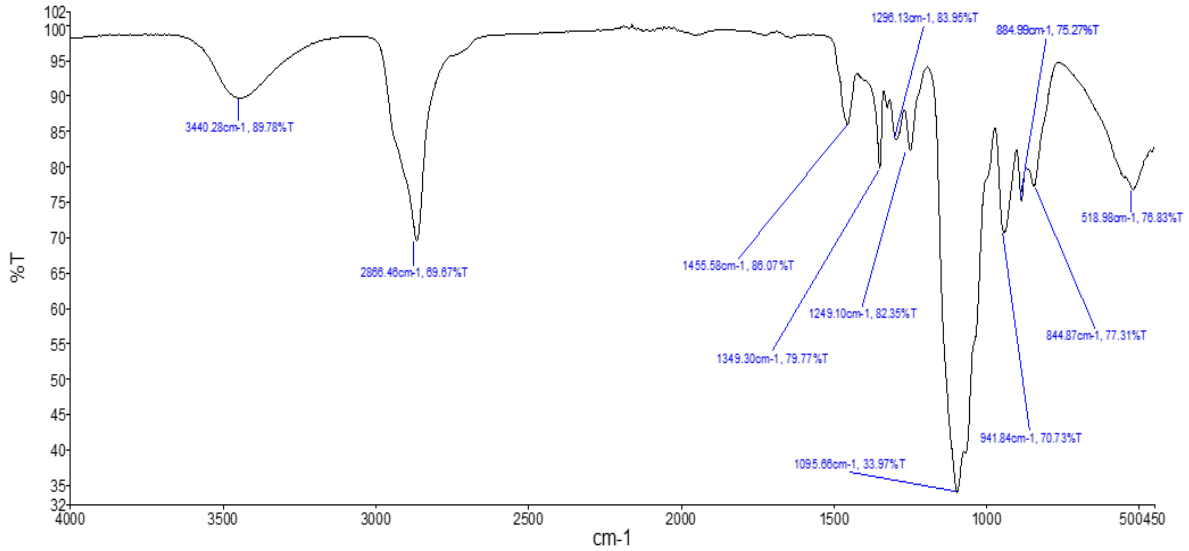


Figure: 1 FTIR analysis of Calotropis procera extract

4.1.2. Preliminary Phytochemical Screening

Calotropis procera leaves extracts were evaluated for the content of alkaloids, glycosides, flavonoids, steroids, terpenoid, cardiac glycosides, phenols, saponins, & tannins using standard techniques. A preliminary phyto-chemical examination of the Calotropis procera extract was done to determine the existence of terpenoids, flavonoids, polysaccharides, alkaloids, anthraquinones, tannins, saponins, glycosides and phlobatamins adopting standard methods.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Phytochemical group	Methanol extract	Chloroform extract	Crude extract
Cardenolides (cardiac glycosides)	Present	Present	Present
Flavonoids (aglycones & glycosides)	Present	Present	Present
Triterpenoids / oxypregnane esters	Present	Present	Present
Sterols	Present	Present	Present
Alkaloids	Present	Present	Present
Saponins	Present	Present	Present
Phenolic acids / tannins	Present	Present	Present
Proteolytic enzymes	Present	Present	Present
Volatile & fatty constituents	Present	Present	Present
Miscellaneous resins & simple glycosides	Present	Present	Present

4.1.3. Antibacterial evaluation

The methanol-based extract of Calotropis procera was examined for its effect on pathogenic micro-organisms. Calotropis procera may have anti-bacterial effects. The extract is more effective towards Bacillus subtilis compared to other bacteria tested, but Klebsiella pneumonia exhibited the smallest degree of action. In the context of this particular trial, Calotropis procera essential oil shown high activity. The standard anti-oxidant compound L-ascorbic acid (Vitamin C) exhibited the maximum free-radical scavenging activity (RSA) of 91.00 ± 1.08 % and the test solution i.e. methanol-based extract of Calotropis procera exhibited the maximum anti-oxidant activity of 77.67 ± 1 %.

4.2. Characterization of Loaded Emulgel

All the six formulations of nanoemulsion as described in table 3.1, were checked thermodynamically stability. The formulation 5 (F5) was more stable and selected for converting emulgel. As mentioned above 2 % Carbopol was prepared separately in beaker and then mix in 1:1 with nanoemulsion formulation. The prepared emulgel was further characterized by using various parameters.

4.2.1. pH of prepared emulgel

The pH value of the newly developed emulgel mixture was evaluated using a digital pH scale, primarily at 0 hours and subsequently across an interval of 28 days. As described in table 4.1, till 28 days the emulgel pH was in the range, while applied 8, 25 and 40 °C temperature regularly.

Table 4.1. pH of prepared emulgel

Days	Temperature (°C)		
	8 (°C)	25 (°C)	40 (°C)
0	5.92 ± 0.76	6.00 ± 1.21	5.94 ± 0.99
1	6.00 ± 0.65	5.99 ± 0.60	5.85 ± 0.84
2	6.02 ± 0.76	5.7 ± 0.99	5.74 ± 0.88
7	6.01 ± 0.87	5.3 ± 0.29	5.62 ± 0.57
14	6.11 ± 0.65	5.2 ± .76	5.47 ± 0.51
28	6.12 ± 0.87	5.10 ± 0.76	5.20 ± 0.72

4.2.2. Thermodynamic stability, viscosity and physical evaluation

Stability studies conducted as per the ICH found the product to be physically and chemically stable, as no significant difference was observed. The formulation was subjected to various temperature i.e., 8 °C, 25 °C and 40 °C +70 RH till four weeks (28 days). The physical appearance was checked regarding phase separation, cracking, change in color, odor change, liquification. After centrifugation process (5000-10,000 rpm for 5 min), there was no phase

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

separation occur. The pH was also checked and the results show the pH was in range as suitable for skin.

4.2.3. FTIR study

The formulations of emulgel was checked via FTIR to check the compatibility and interaction among utilized ingredients. The peaks at 3391.56 cm^{-1} and 2923.66 cm^{-1} show the existence of OH bending alcohol and CH stretching groups, respectively. The 1644.37 cm^{-1} and 1457.01 cm^{-1} pea ks show C=C strectch and -C-H- bending groups, respectively. 1350.15 cm^{-1} peaks show C-H rock alkane group. Among the various components of pharmaceutical dosage forms, the interaction and compatibility of ingredients and also the formulations were checked by FTIR.

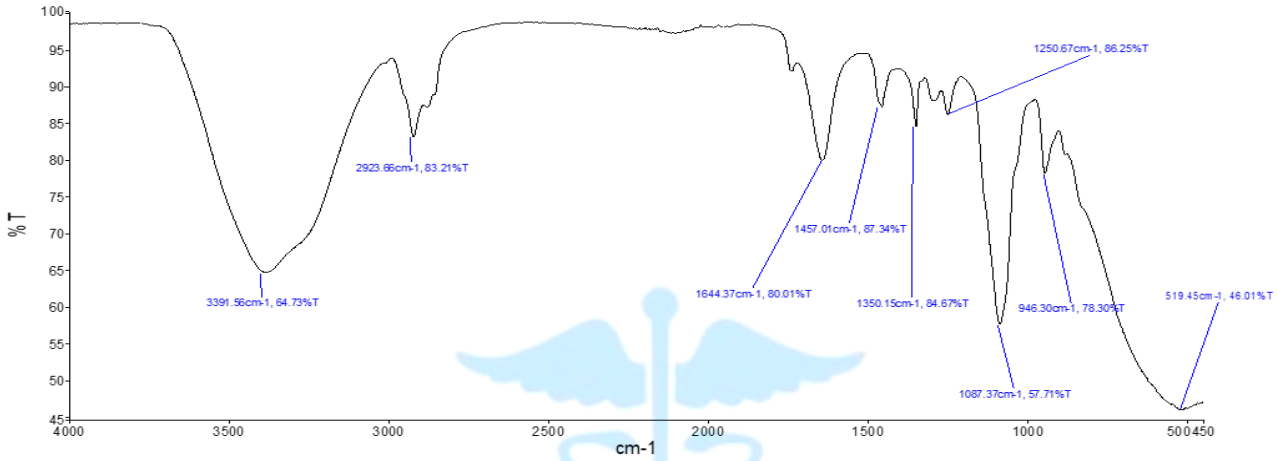
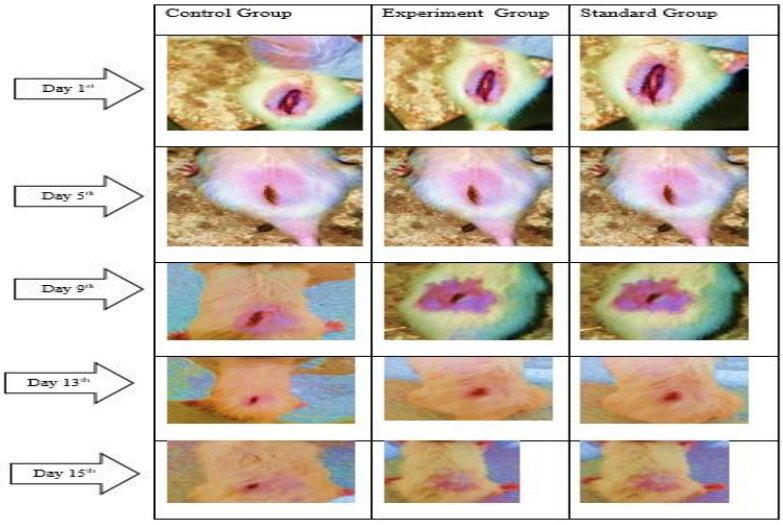


Figure: 4.2. Loaded-Emulgel FTIR

4.3. In vivo wound healing activity

To evaluate wound healing activity of Calotropis procera extract loaded emulgel, rats were used. From ethical review board, the permission was granted to me from ethical review board of Gomal university. First of all, the rats were anesthetized via using injections of xylazine and ketamine in combination. After anesthesia the rats were shaved and induce cut wound in dorsal region of rats. Three groups i.e., Control group, Experimental group and Standard group were kept in separate cages. After completing the 1g treatment protocol of 15 days, the healing of wound of all groups were compared (figure 3).



Fgure: 3. In-vivo study of rats by using loaded-emulgel

Volume 3, Issue 3, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

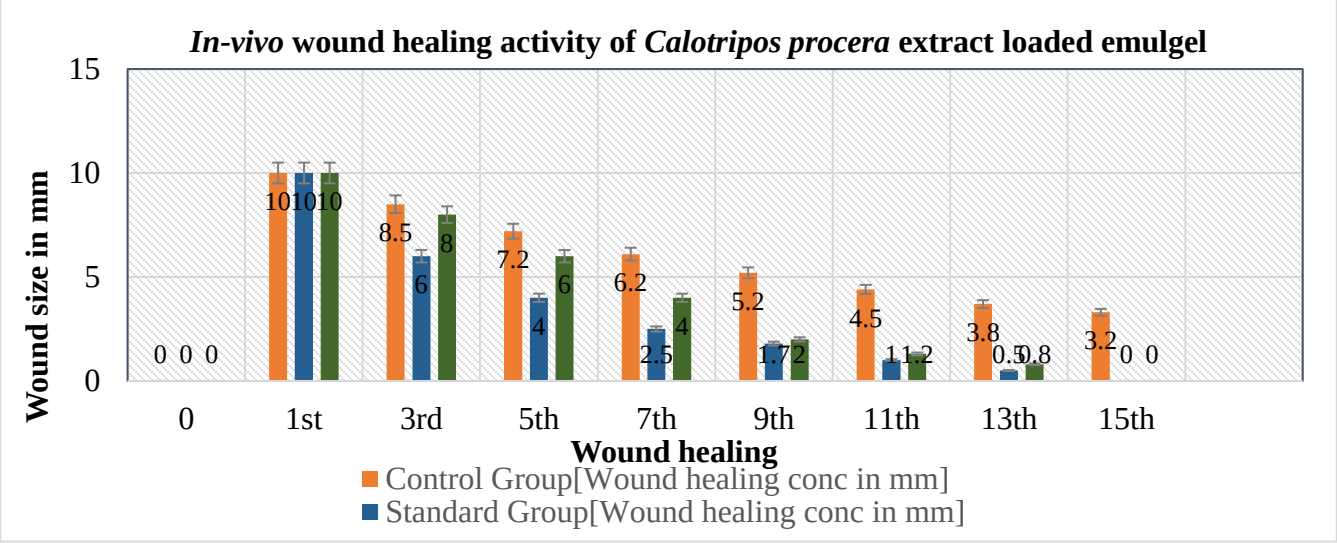


Figure: 3. Graphical representation of In-vivo wound healing activity.

Discussion

The present study highlights the significant antibacterial, antioxidant, and wound healing potential of Calotropis procera extracts. The methanolic and chloroform extracts exhibited strong antibacterial activity, particularly against Staphylococcus aureus and Bacillus subtilis, supporting earlier findings that C. procera contains potent antimicrobial compounds such as flavonoids, terpenoids, and cardenolides [15-16-17]. The chloroform extract's higher efficacy suggests that non-polar bioactives are more responsible for this effect. Additionally, the extract demonstrated robust antioxidant activity in the DPPH assay (77.67%), indicating the presence of phenolic compounds known to neutralize free radicals and reduce oxidative stress, which can otherwise impair wound healing [18]. These pharmacological activities provide scientific support for the plant's traditional use in treating infections and inflammatory conditions [18]. The formulated emulgel exhibited ideal characteristics for topical application, including favorable pH, high viscosity, and excellent stability. FTIR analysis confirmed compatibility between the extract and formulation components, ensuring the product's safety and consistency [19]. The in vivo wound healing study demonstrated that the C. procera-based emulgel significantly enhanced wound contraction compared to the control and standard treatment groups, suggesting the extract promotes fibroblast activity, collagen synthesis, and tissue regeneration (Pandey et al., 2016). Histopathological analysis further confirmed improved re-epithelialization and reduced inflammation. Collectively, these findings suggest that C. procera possesses promising therapeutic value and could be developed into a safe and effective herbal formulation for managing infected wounds.

Conclusion

The extract was successfully prepared by using rotary evaporator. FTIR and phytochemical analysis was performed for the extract. The phytochemical analysis confirmed the presence of various compounds by using standard procedures, i.e., flavonoids, terpenoids, carbohydrates, alkaloids, tannins, saponins, anthraquinones, phlobatamins, and glycosides. Anti-oxidant activity was also performed regarding the methanol-based extract of Calotropis procera which exhibited the maximum anti-oxidant activity of 77.67 ± 1 %. The emulgel formulation of loaded extract was seemed stable thermodynamically and all the characterization of prepared emulgel were performed successfully. The formulation was subjected to various temperature i.e., 8 oC, 25 oC and 40 oC +70 RH till four weeks (28 days). The physical appearance was checked regarding phase separation, cracking, change in color, odor change, liquification, and consistency pH of the formulation was in the range as the skin pH.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

The in-vivo study was performed by using rats. All three groups were checked till 15 days and the result of extract loaded emulgel was satisfactory.

Future Prospective

Perform extensive clinical trials to assess the safety, effectiveness, & pharmacokinetics of Calotropis procera leaves extract-loaded emulgel. Design advanced pharmaceutical delivery methods, including nanoemulsion or liposomal formulations, nanoparticles etc., to increase the extract's stability, therapeutic efficacy and bioavailability. Explore the molecular mechanisms impacted by phytochemicals in Calotropis procera in order to clarify their involvement in wound healing and other biological processes.

REFERENCES

Cordell, G. A. (2011). Sustainable medicines and global health care. Planta medica, 77(11), 1129-1138.

World Health Organization. (2023). Managing epidemics: key facts about major deadly diseases. World Health Organization.

Ahmad, A., Hadi, F., & Ali, N. (2015). Effective phytoextraction of cadmium (Cd) with increasing concentration of total phenolics and free proline in Cannabis sativa (L) plant under various treatments of fertilizers, plant growth regulators and sodium salt. International journal of phytoremediation, 17(1), 56-65.

Houghton, P. J. (1995). The role of plants in traditional medicine and current therapy. The Journal of Alternative and Complementary Medicine, 1(2), 131-143.

Winston, K. R., French, B., & Ketch, L. L. (2023). Skin: Anatomy and Healing. In Plastic Neurosurgery: Opening and Closing Neurosurgical Doors in Adults and Children (pp. 3-37). Cham: Springer International Publishing.

Myers, T. W. (2009). Anatomy trains: myofascial meridians for manual and movement therapists. Elsevier Health Sciences.

Whitney, J. D. (2005). Overview: acute and chronic wounds. Nursing Clinics, 40(2), 191-205.

Boateng, J. S., Matthews, K. H., Stevens, H. N., & Eccleston, G. M. (2008). Wound healing dressings and drug delivery systems: a review. Journal of pharmaceutical sciences, 97(8), 2892-2923.

Vitale, S., Colanero, S., Placidi, M., Di Emidio, G., Tatone, C., Amicarelli, F., & D'Alessandro, A. M. (2022). Phytochemistry and biological activity of medicinal plants in wound healing: an overview of current research. Molecules, 27(11), 3566.

Jung, H., Lee, Y. J., & Yoon, W. B. (2018). Effect of moisture content on the grinding process and powder properties in food: A review. Processes, 6(6), 69.

Chien, F., Sadiq, M., Nawaz, M. A., Hussain, M. S., Tran, T. D., & Le Thanh, T. (2021). A step toward reducing air pollution in top Asian economies: The role of green energy, eco-innovation, and environmental taxes. Journal of environmental management, 297, 113420.

Geetha, T. S., & Geetha, N. (2014). Phytochemical screening, quantitative analysis of primary and secondary metabolites of Cymbopogan citratus (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. International Journal of pharmtech research, 6(2), 521-529.

Cedillo-Olivos, A. E., Juárez-Chairez, M. F., Cid-Gallegos, M. S., Sánchez-Chino, X., & Jiménez-Martínez, C. (2024). Natural preservatives used in foods: a review. Critical Reviews in Food Science and Nutrition, 1-17.

Daud, N. S., Akbar, A. J., Nurhikma, E., & Karmilah, K. (2018). Formulation of snail slime (Achatina Fulica) anti-acne emulgel using tween 80-Span 80 as emulsifying and HPMC as Gelling Agent. Borneo Journal of Pharmacy, 1(2), 64-67.

Al-Mustafa, A. H., & Al-Thunibat, O. Y. (2008). Antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes. Pakistan Journal of Biological Sciences, 11(3), 351–358.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Kumar, S., Malhotra, R., & Kumar, D. (2015). Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacognosy Reviews, 9(17), 88–92.

Pandey, A., Tripathi, S., & Bhattacharya, P. (2016). Wound healing activity of Calotropis procera latex in albino rats. Journal of Pharmacy Research, 10(2), 60–65.

Raut, R. W., Karuppayil, S. M., & Bhalerao, S. A. (2017). Studies on the formulation and evaluation of herbal emulgel of Curcuma longa for wound healing. International Journal of Pharmaceutical Sciences Review and Research, 44(2), 1–6.

Rizwan, K., Zubair, M., Rasool, N., Riaz, M., Zia-ul-Haq, M., &