WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 17, 600-612. Review Article ISSN 2277– 7105

EVALUATION OF WOUND HEALING POTENCY OF '*CASSIA AURICULATA LINN*' FLOWER EXTRACT USING IN-VITRO METHOD

Dr. Shambhulingaiah H. M.*, **Mounika S., Akshtha MJM., Anusha K., Neenu Raj, Ranjith Kumar P. B.**

Associate Professor Dept of Pharmacology, SCS College of Pharmacy, Harapanahalli-583131, Vijayanagara.

Article Received on
01 November 2022,
Revised on 22 Nov. 2022,
Accepted on 12 Dec. 2022
DOI: 10.20959/wjpr202217-26544

***Corresponding Author**
Dr. Shambhulingaiah H. M.
Associate Professor Dept. of Pharmacology, SCS College of Pharmacy, Harapanahalli-583131, Vijayanagara.

ABSTRACT

Wounds are 'The Silent Epidemic' Wounds have a variety of causes; some arise from surgical intervention, some are the result of injury, and others are a consequence of extrinsic factors, such as pressure or shear, or underlying conditions such as diabetes or vascular disease. Wound healing mechanism is obligatory to regain the lost tissue and maintain tissue homeostasis. New tissue formation is a complex process, which involves multiple steps such as inflammation, angiogenesis, granulation tissue formation, re-epithelialisation, and ECM reconstruction. Fibroblasts are most abundant cells in skin tissue and the major functions of these cells during wound healing include, rupturing of fibrin clots, generation of extracellular matrix (ECM) components and collagen structures that support the tissue homeostasis. In vitro cell based scratch assay is an appropriate and inexpensive method for initial understanding of wound healing potential of medicinal plant extracts. The current study was aimed at investigating the wound healing capacity of hydroalcoholic flower extracts of Cassia auriculata (L.) by using scratch assay as a primary model, where proliferative and migratory capabilities of test compounds could be monitored through microscopy studies. A scratch assay involves growing a cell monolayer to confluence in a multiwall assay plate; creating a 'wound'—a cell-free zone in the monolayer— into which cells can migrate; and monitoring the recolonization of the scratched region to quantify cell migration, In our study, we observed that L929 cells migrated better towards the artificially created wound when treated with the *Cassia auriculata* flower extract. This suggests that the extract accelerates wound healing by

inducing the migration of fibroblasts, may be due to the presence of various phytoconstituents present in it.

KEYWARDS: Wound healing, Fibroblast cells, Scartch assay, *Senna auriculata.*

1. INTRODUCTION

Wounds are defined as injury to living tissue which leads to the disruption of its normal anatomical structure and function.[1] They arise due to physical, chemical, thermal, microbial or immunological damage to the tissue.[2] Regardless of the aetiology and type, wounds can cause damage to the tissue and disrupt the surrounding environment. The damage can affect the integrity of the skin epithelial layer and can also extend into the subcutaneous tissue disrupting other structures such as tendons, muscles and nerves. [3] Failure of wounds to heal normally leads to chronic wounds. Chronic wounds are a silent epidemic claiming the lives of many individuals worldwide. In the past decade, it has been estimated that 6 million people suffer from chronic wounds worldwide.[4,6]

Wounds have a significant negative impact on the economic and social lives of patients and their families. They cause severe pain, physical disability such as immobility and loss of function, loss of self-esteem, depression and anxiety as well as premature death, the prevalence of chronic wounds in the community was reported as 4.5 per 1000. population where as that of acute wounds was nearly doubled at 10.5 per 1000 population.

Wound healing mechanism is obligatory to regain the lost tissue and maintain tissue homeostasis. New tissue formation is a complex process, which involves multiple steps such as inflammation, angiogenesis, granulation tissue formation, reepithelialization, and ECM reconstruction.[7] Upon injury in the skin, cells such as fibroblasts, keratinocytes, macrophages, and other immune cells rapidly proliferate and migrate towards the wound and initiate the complex healing process. Hence, migration of cells towards wound is one of the key phases of wound healing process and in general, it is governed by various stimulatory factors of tissue microenvironment. Fibroblasts are most abundant cells in skin tissue and the major functions of these cells during wound healing include, rupturing of fibrin clots, generation of extracellular matrix (ECM) components and collagen structures that support the tissue homeostasis. Collagen synthesis and granulation tissue formation play critical role in wound contraction. For this reason, contemporary wound healing research is focused on the

identification of new therapeutic agents, which has a stimulatory effect on the activation and modification of collagen producing fibroblasts.

Cassia auriculata Linn. commonly known as 'Tanner's Cassia' (Ceasalpinaceae) or *Avartaki* in Ayurveda is an annual or biennial shrub found throughout India in open forests.

The foremost description of the Senna auriculata (L.) Roxb. Is available in Kaiyadeva Nighantu, a classical Ayurveda text (15th century), where its Pramehahara property (antidiabetic action) through different botanical parts of the plant has been mentioned. Flowers have Pramehashamana property (antidiabetic action). Tender fruits have mentioned for their Vamihara (antiemetic), Krimihara (anthelmintic), Sarvapramehahara (antidiabetic), Trishnaghna (thirst alleviating), Akshihita (eye tonic), Ruchya (appetizing) properties. Seeds are useful as Madhumehaghna (antidiabetic), Vishahara (antidote), Raktaatisaraghna (antidiarrheal). Roots are mentioned for Trishnahara (thirst alleviating), Pramehaghna (antidiabetic), Shwasaghna (antiasthamatic), Raktapittashaman (antihemorrhagic), Shukrakshayahara (sperm enhancing) properties.The plant is also having various traditional and ethno pharmacological claims in different parts of India like The leaves of Senna auriculata (L.) Roxb. are also effective in muscle pain, irregular muscle contraction, body pain, gastritis, skin sores and ulcers. The flowers are effectively used as health beneficial agents. The crushed flowers are mixed with goat milk to cure sexually transmitted diseases. The dried powder of flowers of Senna auriculata (L.) Roxb. is used to clean the hair, and taken by diabetic patients and in fever. The root is used by chewing, and the juice is swallowed to cure abdominal complaints, vomiting, urinary discharges, tumors and diarrhea. Powder of bark is used to treat toothache by applying it to the gums. The fruits are used in helminthic infections.[8]

The current study was aimed at investigating the wound healing capacity of hydroalcoholic flower extracts of Senna auriculata (L.) by using scratch assay as a primary model, where proliferative and migratory capabilities of test compounds could be monitored through microscopy studies.

2. Plant Description

Cassia auriculata Linn commonly known as Tanners Senna, is also known as Avaram tree.

Regional and Other Names.[9,10]

English : Tanner's Cassia, Tanner's Senna, Mature Tea Tree

Sanskrit: Avartaki, Pitapuspa, Pitkalika, Manojyna, Pitkala, Charmaranga

Hindi : Tarwar, Awal, Tarval

Kannada: Tangade huvu

Telagu : Tangedu, Merakatangeedu

Marathi : Arsual, Taravada, Tarwad

Botanical Description

Senna auriculata (L.) Roxb. is found in wooden grasslands up to a height of 500 m. It breeds wild in dry regions with annual precipitation of 300 mm. It grows well in areas with an annual temperature range of 15–28°C and needs full sun for its growth. It is a branched shrub with height of 1.5–5 m, trunk diameter of 20 cm and brown lenticellate bark.[11]

Leaves: Leaves are dull green in color, alternate, stipulate, paripinnate arrangement with 16–24 pairs of leaflets. Leaves are narrowly rough, pubescent and thin, with vertical and linear gland between the leaflets (Figure 1). It is short-stalked, 20–25 mm long, 10–13 mm wide; marginally overlapping, rectangular, dull-witted at both ends, and glabrous.

Flowers: Flowers are bright yellow and irregular and large (50 mm) and have axillary raceme inflorescence, 2–8 flowered (Figure 1). Flowers are bisexual, zygomorphic, pentamerous, 4–5 cm; sepals are rounded at apex, imbricate; petals free, imbricate, unequal, 1.5–3 cm long; stamens 10, the 3 lower ones large stand fertile, others usually sterile; ovary superior, falcate, with 1.5 cm long, stalked, style (fruit a flattened) cylindrical pod 5–18 × 1–2 cm, transversely undulate between the 10–20 seeds, indehiscent, green turning to brown when mature. [12]

Fig No 01: Photography of *Cassia auriculata* Linn flower.

3. Materials and methods

The Senna auriculata (L.) flowers Were collected in the month of August to September from various fields of Harapanahalli and authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S College of Pharmacy, Harapanahalli. The herbarium specimen is preserved in S C S College of pharmacy museum.

PREPARATION OF EXTRACT

The flowers of *Cassia auriculata* were collected and washed thoroughly under running tap water and then rinsed in distilled water and allowed to dry for some time. Then the flowers were shade dried without any contamination for about 3 to 4 weeks. The powder was extracted according to standard procedure of soxhelation, The dried flower powdered (coarse) and subjected to Soxhlet appratus (Figure) extract is be prepared by successive soxhlation i.e. extracting dried powder with the solvents of increasing order of polarity i.e. Pet. ether (60-80°), 70% Ethanol (64.5-65.5°) The extraction was done with each solvent until the supernatant in the Soxhlet became transparent for 36 hours. Every time before taking the solvents of higher polarity to remove the traces of previous solvents, exhausted marc was completely dried. Extracts will be concentrated under reduced pressure and stored in airtight container in refrigerator below 10 ^{o}C.

Calculation of percentage yield

The percentage yield was calculated for the extracts with reference to the crude material taken using the formula given below,[13] The percentage yield of the each extract is tabulated in table No.01.

$$\% \text{ yield of extract} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

II. PRELIMINARY PHYTOCHEMICAL SCREENING

The obtained extract will be subjected to preliminary phytochemical screening following the standard procedures described in the practical Pharmacognosy by C.K. Kokate[14] and R.K. Khandelwa,[15] results are summarized in table no.02.

III. IN-VITRO wound healing activity

Fibroblast cell migration/ The scratch wound healing assay[16]

The scratch wound healing assay has been widely adapted and modified to study the effects of a variety of experimental conditions, for instance, gene knockdown or chemical exposure,

on mammalian cell migration and proliferation. In a typical scratch wound healing assay, a"wound gap" in a cell monolayer is created by scratching, and the "healing" of this gap by cell migration and growth towards the centre of the gap is monitored and often quantitated. Factors that alter the motility and/or growth of the cells can lead to increased or decreased rate of "healing" of the gap[17] (Lampugnani, 1999). This assay is simple, inexpensive, and experimental conditions can be easily adjusted for different purposes. The assay can also be used for a high-throughput screen platform if an automated system isused[18] (Yarrow and Perlman, 2004).

MATERIALS

- Cell lines:L929 – Mouse embryo fibroblast cell line (NCCS, Pune)
- Cell culture medium: DMEM high glucose - (#AL111, Himedia)
- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum(#RM10432, Himedia)
- D-PBS (#TL1006, Himedia)
- Test compound: CAFE (cassiua auriculata flower extract)
- Cipladine (Commercially available)
- 12 well cell culture plate (Biolite - Thermo)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000ul tips (TORSON)
- 70% ethanol

EQUIPMENTS

1. Centrifuge (Remi: R-8^{o}C).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted Biological Binocular Microscope (Biolinkz)
4. 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)
5. Biosafety Laminar Hood (Healforce, China)

SOFTWARE

1. Image J
2. Windows Paint

STEPS FOLLOWED

1. Grow cells in DMEM with high glucose media supplemented with 10% FBS until the cells reach 70-80% confluence.
2. Seed cells into 12 well tissue culture plate at a density of 0.25 million cells per well, until they reach ~80-100% confluence as a monolayer for the incubation period of 24hrs.
3. Do not change the medium. Gently and slowly scratch the monolayer with a new 200ulpipette tip across the centre of the well. While scratching across the surface of the well, thelong-axial of the tip should always be perpendicular to the bottom of the well.
4. The resultinggap distance therefore equals to the outer diameter of the end of tip. The gap distancecan be adjusted by using different types of tips. Scratch a straight line in one direction.
5. Scratch another straight line perpendicular to the first line to create a cross in each well.
6. After scratching, gently wash the well twice with medium to remove the detached cells.
7. Treat the cells with desired concentrations of given compoundprepared in media and incubate at 37^{o}C with 5% Co_2 in the incubator.
8. Grow cells for additional 48hours(or the time required if different cells are used).
9. Capture thecell images at different time intervals (ex: 0, 24hr, 48hr., etc)
10. Set the same configurations of the microscope when taking pictures for different views ofthe monolayer. The gap distance can be quantitatively evaluated using software such as ImageJ. To reduce variability in results, it's suggested that multiple views of each well should be documented, and each experimental group should be repeated multiple times.

FORMULA USED FOR THE ANALYSIS:

% of Wound Healing Scored= (Initial area-Final area)/Initial area *100

RESULTS

I. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Percentage yield of crude extract of ***Cassia auriculata flower extract*** is shown in table No 01, Results of the preliminary phytochemical investigation on ***Cassia auriculata flower extract*** are shown in Table No 02.

Table No 01: Successive soxhlet extraction of *Cassia auriculata flower extract.*

Sl. No.	Solvent	Colour and Consistency	Percentage yield
1	Pet. Ether	Greenish black sticky	1.92%
2	70% Ethanol	Brownish black and non sticky	18.31%

Preliminary phytochemical screening of *Cassia auriculata flower extract*

It is observed from the preliminary photochemical screening of the ***Cassia auriculata flower extract*** that glycoside, flavonoid, tannin, alkaloid, saponin, protein and carbohydrate found in 70% ethanolic extract. All are absent in petroleum ether and, It was qualitatively observed that 70% ethanolic extract contain higher concentration of polyphenol, flavonoid, alkaloid, protein and tannin components and previous literature revels the same hence 70% ethanolic extract selected for further study.

Table No. 02: II. phytochemical constituent of *Cassia auriculata flower extract.*

Types of Phytochemical constituents	Petroleum ether Extract	70/ Alcoholic Extract
Alkaloids	+	+++
Carbohydrates	+	+++
Flavonoids	+	+++
Glycosides	-	+
Tannins and Poly phenol	–	+++
Protein	+	+
Steroids	+	++
Saponin	-	+

- Absent ++ More clarity

+ Indicates presence +++ Better response

III. IN-VITRO wound healing activity

Fibroblast cell migration/ The scratch wound healing assay

Fibroblast cell migration was induced by CAFE (*cassiua auriculata* flower extract) Activation, proliferation and migration of fibroblasts are the primary steps in wound healing, where multiple cell types and other micro environmental factors are involved. Scratch assay is a widely applied In vitro technique for understanding the wound healing capabilities of medicinally important compounds. In the current study, L929 cells were treated with 125 µg/mL of A. saccata extract for 48hrs. Cell migration at 0, 12, 24, 48 h were captured and wound closure distance was calculated by Image J software. The results indicated that A. saccata leaf extract, at 125 µg/mL, closed the gap created by the scratch by 93.525% in 48 h.

Percentage wound closure at different time intervals in untreated, extract treated and control drug-treated cells have been represented in Fig. 02. CAFE induced the migration of L929 cells resulting in wound closure. In the standard-drug treated cells,99.05% of the gap was closed at 48 h. Fig. 03 shows the microscopic images of untreated, standard drug-treated and extract-treated L929 cells. The photographs show increased cell migration in thel drug-treated cells and extract treated cells.

Table No 03: wound area covered by std and extract.

Wound Area Covered Overlay-L929 cells			
Incubation	**UNTREATED**	**STD**	**EXTRACT**
0 hour	306298.76±1546	306724.49±1091	303735.75±1959
24 hour	279825.26±9998	84921.13±4155	99494.14±2910
48 hour	162065.29±13527	8294.18±2439	9403.63±3878

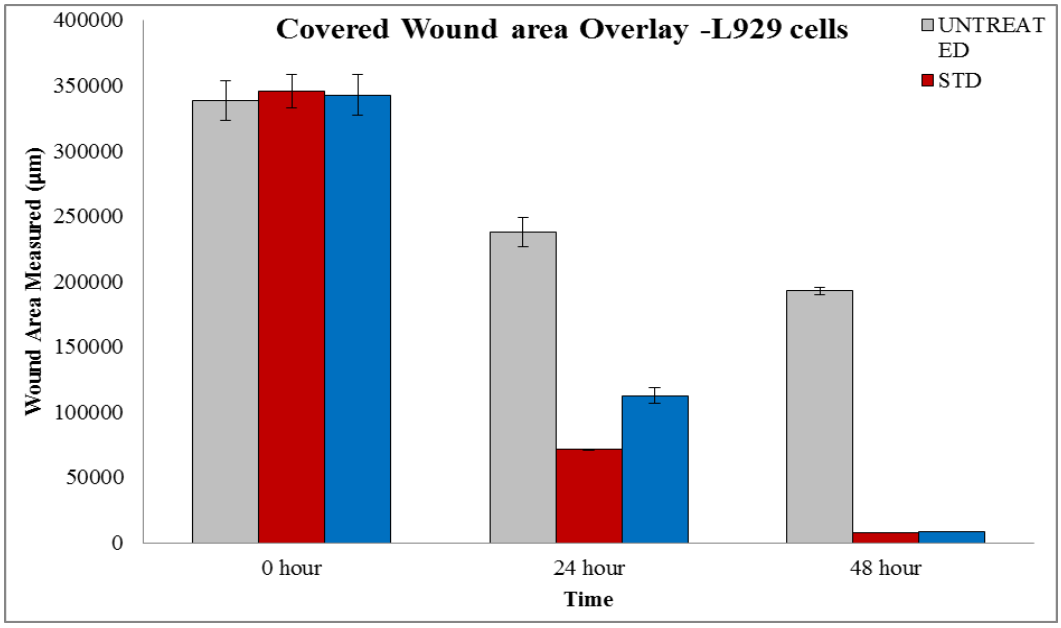
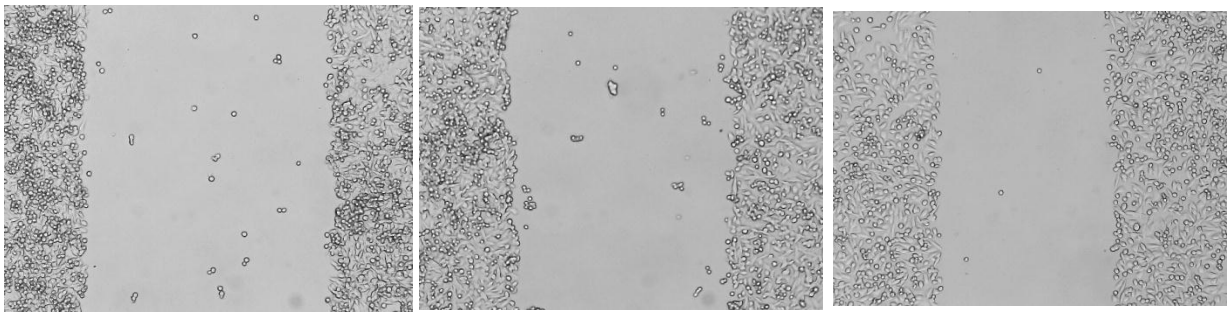


Fig No: 02 wound area covered by std and extract.



EXTRACT-0 HOUR STD-0 HOUR UNTREATED-0 HOUR

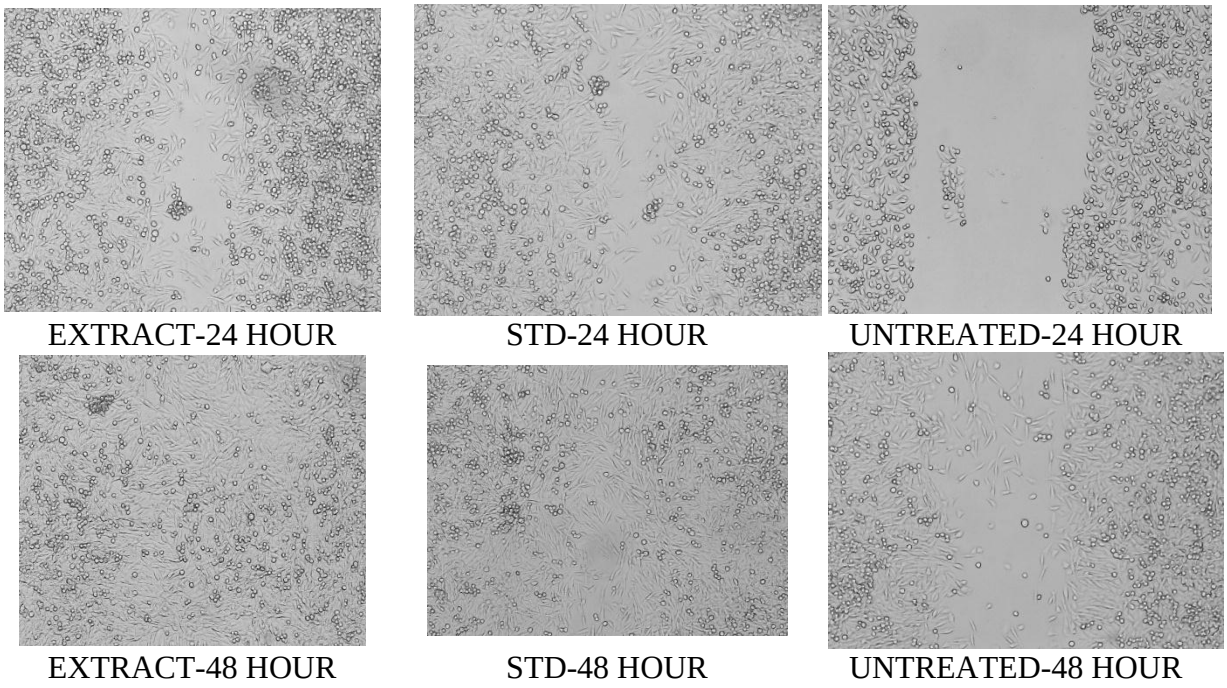


Figure No. 03: Images from a scratch assay experiment at different time points.

IV DISCUSSION

Wound healing mechanism is obligatory to regain the lost tissue and maintain tissue homeostasis. New tissue formation is a complex process, which involves multiple steps such as inflammation, angiogenesis, granulation tissue formation, reepithelialization, and ECM reconstruction.[19] Upon injury in the skin, cells such as fibroblasts, keratinocytes, macrophages, and other immune cells rapidly proliferate and migrate towards the wound and initiate the complex healing process. Hence, migration of cells towards wound is one of the key phases of wound healing process and in general, it is governed by various stimulatory factors of tissue microenvironment. Fibroblasts are most abundant cells in skin tissue and the major functions of these cells during wound healing include, rupturing of fibrin clots, generation of extracellular matrix (ECM) components and collagen structures that support the tissue homeostasis. Collagen synthesis and granulation tissue formation play critical role in wound contraction.

The scratch or wound healing assay is the method of choice for studying cell migration due to the low cost and simplicity of its experimental design,. A scratch assay involves growing a cell monolayer to confluence in a multiwall assay plate; creating a ‘wound’—a cell-free zone in the monolayer— into which cells can migrate; and monitoring the recolonization of the scratched region to quantify cell migration, In our study, we observed that L929 cells

migrated better towards the artificially created wound when treated with the CAFE. This suggests that the extract accelerates wound healing by inducing the migration of fibroblasts.

This indicates that CAFE as potential wound healing properties and can be used to extract lead molecules in the discovery of wound healing agents. The phytochemical analysis of plant extract shows the presens of various phytoconstituents including flavonoids and triterpenoids and also other well-known wound healing plants reveal the possible role of these phytoconstituents in wound healing, the review literature of the plant and ethnobatonical claims shows that plant is having astringent, free radical scavenging and antioxidant properties, which are known to aid wound healing process, [20,21]. Another possible mechanism is that the plant extracts increase the proliferation of fibroblasts cells and in turn increase the production of collagen in the affected area. This may the possible mechanism of wound healing further studies needed to elucidate role of phytoconstituents in increasing the total collagen content of granulation tissues in wounds.

V. CONCLUSION

In conclusion, the CAFE enhanced wound closure in L929 cells and. Further,by literature studies the extract was found to have no cytotoxic effect. These data suggest that CAFE has possible wound healing properties and can be a plausible source for the extraction of natural wound healing compounds

ACKNOWLEDGEMENT

We are thankful to all the authors for providing the information and also we are thankful to the management and all the staff members of SCS College of pharmacy Harapanahalli.

REFERENCE

1. Lindholm C, Searle R. Wound management for the 21st century: combining effectiveness and efficiency. Int Wound J, 2016; 13 (suppl. S2): 5–15.
2. Caroline Tyavambiza, Phumuzile Dube , Mediline Goboza, Samantha Meyer, Abram Madimabe Madiehe and Mervin Meyer. Wound Healing Activities and Potential of Selected African Medicinal Plants and Their Synthesized Biogenic Nanoparticles. Plants, 2021; 10: 2635.
3. Abubacker, M.N.; Devi, P.K. Evaluation ofWound Healing Activity of Ethanolic Extract of Lepidagathis Cristata. Willd. (Acanthaceae) in Experimental Animal models. Eur. J. Pharm. Med. Res, 2016; 3: 410–416.

4. Kayode, A.O. Epidemiological Study on Wound Distribution Pattern in Horses Presented at Two Veterinary Clinics in South West, Nigeria between 2007–2010. J. Dairy Vet. Anim. Res, 2017; 5: 127–129.
5. Barku, V. Wound Healing: Contributions from Medicinal Plants and Their Phytoconstituents. Annu. Res. Rev. Biol, 2018; 26: 1–14.
6. Agyare, C.; Boakye, Y.D.; Bekoe, E.O.; Hensel, A.; Dapaah, S.O.; Appiah, T. Review: African medicinal plants with wound healing properties. J. Ethnopharmacol, 2016; 177: 85–100.
7. Kumar, B.; Vijayakumar, M.; Govindarajan, R.; Pushpangadan, P. Ethnopharmacological approaches to wound healing—Exploring medicinal plants of India. J. Ethnopharmacol, 2007; 114: 103–113.
8. Järbrink, K.; Ni, G.; Sönnergren, H.; Schmidtchen, A.; Pang, C.; Bajpai, R.; Car, J. Prevalence and incidence of chronic wounds and related complications: A protocol for a systematic review. Syst. Rev, 2017; 6: 1–7.
9. Frykberg, R.G.; Banks, J. Challenges in the Treatment of Chronic Wounds. Adv. Wound Care, 2015; 4: 560–582.
10. Zhao, R.; Liang, H.; Clarke, E.; Jackson, C.; Xue, M. Inflammation in chronic wounds. Int. J. Mol. Sci, 2016; 17: 2085.
11. Agale, S.V. Chronic Leg Ulcers: Epidemiology, Aetiopathogenesis, and Management. Ulcers, 2013; 413604.
12. Ibrahim, N.I.; Wong, S.K.; Mohamed, I.N.; Mohamed, N.; Chin, K.Y.; Ima-Nirwana, S.; Shuid, A.N. Wound healing properties of selected natural products. Int. J. Environ. Res. Public Health, 2018; 15: 2360.
13. Marume, A.; Matope, G.; Katsande, S.; Khoza, S.; Mutingwende, I.; Mduluza, T.; Munodawafa-Taderera, T.; Ndhlala, A.R. Wound Healing Properties of Selected Plants Used in Ethnoveterinary Medicine. Front. Pharmacol, 2017; 8: 544.
14. Mustoe, T. Understanding chronic wounds: A unifying hypothesis on their pathogenesis and implications for therapy. Am. J. Surg, 2004; 187: S65–S70.
15. Sabale, P.; Bhimani, B.; Prajapati, C.; Sabale, V. An overview ofmedicinal plants as wound healers. J. Appl. Pharm. Sci, 2012; 2: 143–150.
16. Gupta N, Gupta SK, Singh SP, Shukla VK. A community based epidemiological study of wounds. J Wound Care, 2004.
17. Lampugnani MG, Cell migration into a wounded area in vitro. Methods Mol Biol, 1999; 96: 177-182. 10.1385/1-59259-258-9:177.

18. Justin C Yarrow, Zachary E Perlman, Methodology article Open Access A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods. BMC Biotechnology, 2004; 1-9.
19. Srinivasa Rao Bolla, Abeer Mohammed Al-Subaieb, Reem Yousuf Al-Jindan, Janardhana Papayya Balakrishna, Padma Kanchi Ravi, Vishnu Priya Veeraraghavan, Aruthra Arumugam Pillai, Shiva Shankar Reddy Gollapalli, Joel Palpath Joseph, Krishna Mohan Surapaneni, In vitro wound healing potency of methanolic leaf extract of Aristolochia saccata is possibly mediated by its stimulatory effect on collagen-1 expression. Heliyon, 2019; e01648.
20. O. James, E.T. Friday, Phytochemical composition, bioactivity and wound healing potential of Euphorbia heterophylla (Euphorbiaceae) leaf extract, Int. J. Pharm. Biomed. Res, 2010; 1(1): 54–63.
21. C. Shenoy, M.B. Patil, R. Kumar, S. Patil, Preliminary phytochemical investigation and wound healing activity of Allium cepa Linn (Liliaceae), Int. J. Pharm. Pharm. Sci, 2009; 2(2): 167–175.