JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

FORMULATION AND STANDARDIZATION OF *CALENDULA OFFICINALIS* FOR WOUND HEALING

Gaurang Sharma, Vikash Sharma, Khushboo Lavania *

Affiliation- Anand College of Pharmacy, Keetham, Agra (U.P.), Pin-282007

Corresponding Author

Mr. Vikash Sharma
Assistant Professor
Anand College of Pharmacy, Agra
Email id- vikassharma10588@gmail.com
Contact no. - +91 7999956617

ABSTRACT:

The treatment alternatives are accessible for the administration of most kinds of wounds which intense just as incessant. One operator that has been utilized for a long time for the treatment of dermatological issue and has a numerous quantities of pharmacological activities that are valuable to wound mending that is *Calendula officinalis*, or pot marigold. Home grown plants give a rich source to social insurance to forestall and treat diverse neurotic states. *Calendula officinalis*, the pot marigold, normal marigold or Scotch marigold, is a plant of the family Asteraceae. The fundamental synthetic segments found in the flowers are saponins, triterpenes, liquor triterpenes, unsaturated fat esters, carotenoids, flavonoids, coumarines, basic oils, hydrocarbons, and unsaturated fats. *Calendula officinalis* is a plant that has numerous pharmacological activities like injury mending, aggravation, eye disease, menstrual period issues, ulcer, stomach upset etc. In this investigation, the ethanolic extract methodology is done to acquire the concentrate of calendula officinalis herb just as the tender Thin Layer Chromatography profile of calendula officinalis leaf, blossom and stamps of the plant. This

exploratory examination will uncover that *calendula officinalis* introduced calming properties acting in a positive manner on the provocative of the mending procedure. Extractive value and Ash value also determine the quality of the crude drug and that crude drug ethanolic extract use for the formulation of herbal cream and further evaluation process to be used to test calendula officinalis extract ethanolic herbal cream.

KEY WORDS: Marigold, extraction, *Calendula officinalis L*., scavenging activity.

INTRODUCTION

Calendula, otherwise called *Calendula officinalis*, is a very notable restorative herb. It is local to Northern Mediterranean nations. *Calendula* is a yearly blossom, which allude to the inclination it needs to sprout in like manner with the schedule. *Calendula* is an advanced Latin minute of the word calendae, which signifies "little clock" or "little schedule". It is typically blossoms alongside the full moon or possibly once every month. The more regularly known name, pot marigold or the moniker "Mary's Gold" alludes to the Virgin Mary. *Calendula* likewise was utilized in eighteenth and nineteenth hundreds of years as to add shading to cheddar. Calendula is a bloom that can grow up to 31 inches tall. Its leaves, which are masterminded spirally, can develop from 2 to7 inches long, and are shaggy on the two sides. The blossom itself is regularly brilliant orange, yellow, or gold. [1,2] There are more than 100 assortments of calendula known to exist. *Calendula* herb has an incredible number of employments in a wide range of ventures, including beautifiers. Egyptians take this herb to increment revive power and furthermore in the Hindus world, the individuals respect this herb to satisfy their divine beings in their sanctuaries with the blossom, in light of the fact that the blossoms has a flawlessly shaded and has a lovely smell, calendula has been believed to have the option to secure people.[3,4]

The methods used to make sure that a drug enters the body and goes to the area where it is needed are known as medication delivery systems. Skin prescription association is a restricted drug delivery system that uses the ocular, rectal, vaginal, and skin as a skin course to deliver medication wherever in the body. [5-8] The skin is one of the human body's easiest organs to access quickly, and it plays a crucial role in the organisation of subject drug transport. Topical planning is applied to the skin for the surface, neighborhood or foundational

impact of the medication. Once in a while, the base might be used alone for its remedial properties, for instance, emollient, reducing or protective action. Such huge numbers of topical arrangements contains restoratively dynamic fixing which is scattered or broken up in the base. The blend of dynamic fixings and base gives the extension to a wide scope of topical groundwork for some kinds of medication conveyance and treatment terms used to order the base of topical arrangements in which restoratively dynamic fixings are fused, which might be founded on their physical properties or on their proposed use and piece of the formulation.[9]

A key physiological process called cutaneous injury repair involves the coordinated action of many different cell types and their byproducts. At an early stage of the incendiary stage, attempts are made to repair the damage caused by a local conflict. Finally, they produce repair, which entails the replacement of specific structures made possible by the testimony of collagen, and recovery, which is compared to the process of cell expansion and back separation using pre-existing tissue cells or possibly underlying microbes. These tools don't typically disallow themselves, so following a skin abrasion, in a comparable tissue, recovery and repair may be possible, depending on the cell strains compromised by the injury [10-12]. The mending of a surface injury is advanced by reaching the injury surfaces with a suspension of particles of collagen and a glycoaminoglycan that is chemotactic of fibroblasts as well as endothelial cells [13]. Hemostasis, aggravation, extension, and redesigning are the four precisely and considerably modified stages that the human body uses to carry out turned recovering as a routine natural approach. For a physical issue to retouch adequately, every one of the four phases must occur in the most ideal course of action and time span. Various components can interfere with in any event one times of this system, thusly causing unseemly or incapacitated injury recovering.[14]

In this day and age the interests of home grown beauty care products are expanding step by step. Natural detailing is gathering more focus as a rule in view of their top notch properties and less symptoms. Plus, it is additionally give the skin essential supplements. [15,16] These are the beauty care products which are readied utilizing plant items having restorative activities. The use of botanicals in beauty care products has increased recently, largely due to their mild activity and lack of poisonousness. Both natural and phyto-fixings are used

in cosmetics. Items like oils, removes, emissions, and so forth are common. Phyto-fixings include pure components obtained through various processes. [17,18]

This study examines the phenolic components in successively extracted marigold extracts using thin-layer chromatography (TLC) as well as the scavenging potential of these extracts.

MATERIALS AND METHODS:

Calendula officinalis was collected from Dr. Willmar Schwabe, India (Nature for Health) World Largest Manufacturer of Homeopathic Medicines, from Germany.

Tinctures are alcoholic or hydro-alcoholic solutions usually containing comparatively low concentrations of active principles of vegetables drug. Certain alcoholic solutions of chemicals were previously known as tinctures.[19]

For preparing tincture, we use Maceration Process.

Firstly, we take 3 types of *calendula* crude drug product and dried it in UV rays and then separate the stem and flowers of calendula. Take 100gm of all the 3 types of drug products in 3 different containers. Now fill 600ml of distilled water in all the 3 containers and also add 437ml of ethanol in all the 3 containers and make them air tight. And then place it for 15 days with tightly air packed containers in cool and dark place. After 15 days, filtrate the solution and the 3 types of tinctures were prepared.

Fig -1: Tincture prepared by maceration process Represented in Fig no. 1

The natural creams are mixtures that contain phytochemicals from various herbal sources, which have an impact on the skin's elements and provide essential nutrients for healthy skin.[20]

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a significant procedure for ID and partition of blends of natural mixes. It is helpful in Identification of segments of a blend investigating divisions gathered during cleaning.[21]

Procedure:

Firstly we took 3 pre-covered TLC plate and apply specks of the examples on 3 distinct plates. Now permit it to dry in ordinary condition. Afterthat, the dissolvable framework was readied utilizing Benzene: Ethanol: Chloroform in 1:1:1, 2:1:1, 1:2:1 proportion. Now the TLC plate was moved into the dissolvable chamber and conceals the upper bit of the chamber and the dissolvable go through the plate. First the separation that the dissolvable runs was controlled by utilizing the scale. Then the plate was set in the UV cupboard for the identification of the example tops. Now the plate was resolved in 254nm and 366nm sporadically.[22-24]

BENZENE: EHANOL: CHLOROFORM in 1:1:1, 2:1:1, 1:2:1 proportion

Preparation of Cream:-

Cream is dividing into two types: - Oil in water (o/w) and Water in oil (w/o) .In this arrangement we are use water-in-oil type base in light of the fact that numerous cream-fused medicines are hydrophobic and will be discharged from a water-in - oil cream more quickly than an oil-in - water cream..Alternatively, w/o cream is all the more saturating as it offers a sleek barrier that removes water from the most visible layer of skin by using three different quantity of sample 1 of *C.officinalis* extract for the formulation of herbal cream, there are three herbal cream formulation were prepared. [25] Procedure: - A. Preparation of cream base:- • All ingredients should be weighted accurately. • Bees wax melted into a porcelain dish and then methyl paraben and propyl paraben were added into the melted base. • After homogenization, polysorbate80, ascorbic acid, olive oil and stearyl alcohol were added into above melted base. • Borax should be dissolved into sufficient quantity of water and it should be warmed. [26]• Then the borax water should added drop by drop with

vigorous stirring into the oily portion. • Then melted mass should be allow to cool to get desire consistency. B. Preparation of *C. officinalis* extract herbal cream:-The extract of *C.officinalis* weight accurately and homogenize it properly. • The resulted mass should be added into the base with constant stirring.[27] • Then the perfume and rose oil also added in the formulation as flavouring and fragrance properties.

Cream formulation represented in Table No. 1

TABLES

Table no. 1 Cream Fromulation

FORMULA % W/W					
Sr.No.	**Ingredients**	**A**	**B**	**C**	**Role Of Ingredient**
1	Stearyl Alcohol	3.5	3.5	3.5	Thickeing Agent
2	Borax	0.05	0.05	0.05	Preservative
3	Beeswax	5	5	5	Cream Base
4	Rose Oil	0.8	0.8	0.8	Flavouring Agent
5	Oilve Oil	11.6	11.6	11.6	Moisturing Vehical
6	Polysorbate 80	40.75	40.75	40.75	Emulsifying Agent
7	Methyl Paraben	0.1	0.1	0.1	Antibacterial Agent
8	Propyl Paraben	0.2	0.2	0.2	Antifungal Agent
9	Ascorbic Acid	4	4	4	Antioxidant Agent
10	Calendula Extract	20	17	14	Healing Agent
11	Water	q.s upto 50gm	q.s upto 50gm	q.s upto 50gm	Vehicle

RESULT

Table of TLC for Ratio 1:1:1 Represented in Table no. 2

Table no.2 TLC data ratio 1:1:1

Sample No.	**Rfa**	**Rfb**	**Rfc**	**Rfd**	**Rfe**	**Average**
Sample1	1	0.84	0.82	0.78	0.96	0.88
Sample2	0.87	0.87	0.90	0.85	0.93	0.88
Sample3	0.91	0.9	0.88	0.9	0.86	0.89

Table of TLC for Ratio 2:1:1 Represented in Table no. 3

Table no.3 TLC data ratio 2:1:1

Sample No.	Rfa	Rfb	Rfc	Average
Sample1	0.85	0.72	0.87	0.81
Sample2	0.71	0.66	0.77	0.71
Sample3	0.66	0.76	0.85	0.75

Table of TLC for Ratio 1:2:1 Represented in Table no. 4

Table no.4 TLC data ratio 1:2:1

Sample No.	Rfa	Rfb	Rfc	Rfd	Average
Sample1	1.01	0.92	0.90	0.73	0.89
Sample2	0.87	0.88	0.85	0.79	0.84
Sample3	0.89	0.96	0.81	0.89	0.88

Fig Identification of drug by TLC observed sample Peaks for ratio 1:1:1 Represented in Fig no. 2

Fig Identification of drug by TLC observed sample Peaks for ratio 2:1:1 Represented in Fig no. 3

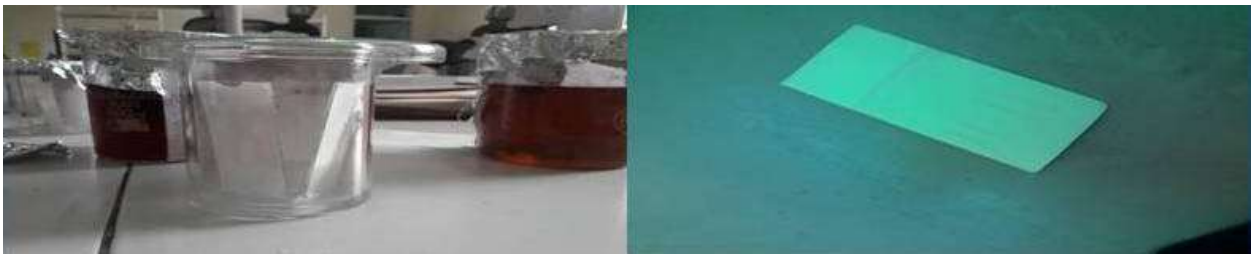

Fig Identification of drug by TLC observed sample Peaks for ratio 1:2:1 Represented in Fig no. 4

EVALUATION PARAMETERS

pH Test

The pH of the cream was determined using a pH metre and corrected using a common buffer solution. In 50.0 ml of distilled water, 0.6 g of cream was measured, broken up, and its pH was calculated.[28-29]

Table: pH Evaluation parameter of all formulation Represented in Table no. 5

Table-5: pH Evaluation parameter of all formulation

Sr.No.	Evaluation Parameters	Acceptance Criteria	A	B	C
1	pH	4.5-6	6.1	5.7	5.6

Evaluation related to pH in table 6.6 All the three batches exhibit value is inacceptable criteria.

Spreadability Test

Utilizing a spreadability device determined the product's spreadability. Two glass slides (7.5 x 2.5 cm each) compensate the mechanical assembly. One of the slides was anchored to the wooden board, and the other was mobile and tied to a rope that ignored a pulley and carried a weight. Between the two glass slides, 1 g of the product was placed. To remove the tangled air between the slides and produce a uniform film of the detailing, a 100 gramme weight was allowed to rest on the upper slide for a period of one to two minutes.[30] After the weight was removed, a force was applied to the top slide over the pulley to expose it to the force. The amount of time needed for a sliding slide to move 6.5 cm apart was noted. The findings showed that different formulations had varying degrees of spreadability.[31-33]

S = M.L/T

Where, M= weight attached to upper slide (30)

L= Length of glass slides (6.5cm)

T= Time taken to isolate the slides.

Table: Spreadability Evaluation parameter of all formulation Represented in Table no. 6

Table-6: Spreadability Evaluation parameter of all formulation.

Sr.No.	Evaluation Parameters	Acceptance Criteria	A	B	C
1	Spreadability	8.50-11.50	5.5	10.8	10.8

Table: Spreadability Test Data Represented in Table no. 7

Table-7: Spreadability Test Data.

Sample No.	Time (sec)	Spreadability (g.cm/sec)
A	35	5.5
B	18	10.8
C	18	10.8

Antimicrobial Test:

Convention: The Nutrient agar media was utilized. *Staphylococcus aureus* microorganism culture was utilized. Incubation time was arrangement for 24hrs. [35]

Strategy: Agar bore well diffusion method.

Technique: *Staphylococcus aureus* (Gram +ve microorganisms) suspension was presented in each plates and 40ml of clean supplement agar media was filled each disinfected plates. The plates were agitated cautiously to permit a homogenous blending of the agar with the test organism. The plates were left on the flat strong surface and permit to solidify. In each plate 1cup, 10mm in diameter was exhausted in the medium with plug borer. [36] The plates of agar were evacuated by cleaned analyzing needle while being mindful so as not to harm the cups. In each plate equivalent sum 0.30gm of cream formulation having same quality was set in the

cup and the plates were brooded at37oC± 2oC for 24hrs.in hatchery. The whole activity was completed under aseptic condition and zone of hindrance was determined.[37,38]

The antimicrobial study of herbal cream formulation shown that all the three batches of herbal calendula cream formulation possess good zone of inhibition. However, Sample C had a higher value of zone of inhibition. (2.5cm). Hence considered better than sample A and B.[39]

Table: Zone of inhibition of *calendula* herbal cream Represented in Table no. 8

Table-8: Zone of inhibition of *calendula* herbal cream

Sr. No.	Sample	Quantity of Sample	Zone of inhibition Diameter (cm)
1	Sample A	0.30 gm	1.0
2	Sample B	0.30 gm	1.4
3	Sample C	0.30 gm	2.5

Viscosity Test

The Brookfield Viscometer has been used to determine the natural herbal formulation's viscosity. The consistency assurance was finished utilizing Brookfield DV-II + viscometer utilizing LV-4 shaft. The herbal formulation is filled in the connector of the viscometer and the rakishly speed expanded steadily from 0.5 to 20 rpm. [41]

The viscosity of the home grown cream result was appeared in the scope of 500-1000 cps which insures that the cream is effectively and easily spreadable on the outside of the skin by utilizing little measure of shear. Detailing B and C has indicated slight edge than A. [42,43]

Homogenesity Test

The herbal formulation's homogeneity was tested both visually and physically. The after effect of the considerable number of parts of home grown cream formulation makes consistently circulation of the concentrate in the cream. By contact and outward appearance, this was insured.[44]

DISCUSSION:

This study is aimed for improving and evaluating *Calendula.officinalis* herbal cream for the treatment of wound healing. During this study, three samples of *Calendula officinalis* creams were prepared by incorporating three different quantities of *Calendula officinalis* extract. The active constituents present in *C. officinalis* extract were identified as 0.88 & 0.89 by using the procedure of Thin layer chromatography. Evaluation studies were performed on all three samples of the Calendula cream formulations, and the evaluation metrics of the formulations were compared.

Studies of Pre-formulation implied that the stability of all the excipients used in the formulation is safe and stable. No physical, chemical or therapeutic incompatibility was discovered in the donation research between drug and the excipients. Hence no harmful effects were noticed with the drug extract as well as with the excipients.

pH test, spreadability test, anti-microbial test, irritancy test, dye test, viscosity test, and homogeneity test evaluation parameters of all three samples of the formulation were determined. The results of evaluation parameters exhibit excellent stability and wound healing quality of *C. officinalis* herbal cream formulation.

The studies of mechanism of action of wound healing capacity of *C.officinalis* have shown a slight better edge of Sample-C over sample A and B.

The Brookfield Viscometer is used to measure the viscosity of the herbal mixture. The viscosity of the herbal cream was 500–1000 cps, which ensures that the cream can be applied to the skin's surface smoothly and readily with only a small amount of shear. Formulation C and B shown slightly better spreadability property than formulation A.

The uniformity of the herbal formulation was examined visually and physically. The results indicate all the components of herbal cream formulation were uniformly dispersed along with the extract in the cream. This was guaranteed by both touch and appearance.

Using a pH metre that had been calibrated with a standard buffer solution, the pH of the cream was determined. The pH of the cream was determined after it had been weighed 0.6g and diluted in 50.0 ml of

distilled water, and its pH was measured. All the three samples exhibit values within the acceptable criteria range.

The result of this formulation of dye test is shows disperse globules appears red and continuous phase appears colorless which clearly state that the Formulation is w/o type emulsion.

The presence of irritability, erythema, and edoema was monitored for up to 24 hours. During irritancy test trials, none of the three samples of calendula cream formulations exhibited any redness, edoema, inflammation, or irritation.

The antimicrobial study of herbal cream formulation shows that all the three batches of herbal calendula cream formulation possess good zone of inhibition. Sample C had shown slightly higher value of zone of inhibition (2.5cm) than sample A and B.

CONCLUSION:

The prepared *C. officinalis* w/o herbal cream for wound healing (Batch A, B, and C) exhibit good organoleptic properties. The results of all evaluation criteria are outstanding, demonstrating that the healing is obvious and that the infection process' progress has been halted. Based on the results of wound healing capacity and evaluation parameters of all three samples of *Calendula* formulations, Sample C (with Calendula extract concentration 14 gm.) has shown better results than sample A and B.

In conclusion, Sample C stood as the improved formulation of *Calendula* cream.

This cream aids in the reduction of itchiness, redness, pain, dryness, the fading of various scars, and the loss of hair in the area of the wound. There is no intolerance displayed, and patients are very satisfied. This cream in the category of herbal formulation therefore it does not have any side effect on the surface of the skin.

Study of Preformulation found that the unlimited sort of amount was stable with all the excipients used within the research of the donation. No physical, chemical or therapeutic incompatibility was discovered in the donation research between drug and excipients and no harmful effect were determined between the drug and excipients.

In this investigation, the ethanolic extract methodology is done to acquire the concentrate of *C. officinalis* herb just as the tender TLC profile of *C. officinalis* leaf, blossom and stamps of the plant. This exploratory examination will uncover that *C. officinalis* introduced calming properties acting in a positive manner on the provocative of the mending procedure.

Extractive value and Ash value also determined the quality of the crude drug and that crude drug ethanolic extract use for the formulation of herbal cream and further evaluation process to be used to test *C. officinalis* extract ethanolic herbal cream.

ACKNOWLEDGEMENT:

It gives me a great pleasure to record my deep sense of gratitude and indebtedness to my esteemed guide Dr. M.A.Sheela, M. Pharm., Asst. Professor, KSOP Department of Pharmaceutics, KIET School of Pharmacy, Meerut Road, Delhi NCR.

CONFILCT OF INTEREST

The authors have no conflict of interest for the publication of this work.

REFERENCES

1. Debjit B., Harish G., B. Pragati K, S. Duraivel, K.P.Sampath Kumar "THE PHARMA INNOVATION Recent Advances In Novel Topical Drug Delivery System'' Vol. 1 No. 9 2012 ,Page | 12, ISSN: 2277- 7695.

2. Ana Cristina de Oliveira Gonzalez,Tila Fortuna Costa, Zilton de Araújo Andrade, and Alena Ribeiro Alves Peixoto Medrado, "Wound healing - A literature review", An Bras Dermatol. 2016 Sep-Oct; 91(5): 614–620.doi: 10.1590/abd1806-4841.20164741, PMCID: PMC5087220,PMID: 27828635.

3. Michaeli D, Francisco S, Calif, Horne T.R., Thurman K., "COMPOSITIONS, ARTICLES AND MEHTOD FOR IMPROVING WOUND HEALING", This is a division of Ser. No. 897,103, filed Aug 15, 1986, Patent Number: 4,837,024

4. Dhyani A, Chander V, Singh N, "Formulation and evaluation of multipurpose herbal cream" Journal of Drug Delivery and Therapeutics. 2019; 9(2):341-343.

5. Saudagar R. B. and Sisodiya M. H. "World Journal of Pharmaceutical Research", Vol 7, Issue 7, 2018, Page no. 573-591

6. Singh M, Sharma S, Khokra S.L., Sahu R.K., Jangde R, "PREPARATION AND EVALUATION OF HERBAL COSMETIC CREAM", Pharmacologyonline 2: pg.1258-1264 (2011) .

7. Nicola L. and Luigi R., ''Labeling of Cosmetic Products", Cosmetics 2018, 5, 22; doi: 10.3390/cosmetics5010022.

8. Pan Y., Gerhard L, Gao S, Zhou S., Zhi-Ling Yu, Chen H, Zhang S., Tang M, Jian-Ning Sun, and Kam-Ming Ko "Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources" Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Volume 2014, Article ID 525340, 20 pages.

9. Deosarkar S.S., Khedkar C.D., Kalyankar S.D. and Sarode A.R. (2016) Cream: Types of Cream. In: Caballero, B., Finglas, P., and Toldra, F. (eds.) The Encyclopedia of Food Health vol.2, pp. 331-337. Oxford: Academic press.

10. Nattapong P. and Utai K., "Factor affecting the properties of water-in-oil-in-water emulsions for encapsulation of minerals and vitamins'', Songklanakarin J. Sci. Technol. 36 (6), pg.651-661, Nov. - Dec. 2014.

11. Chicago, S. Paulina Ave., Chicago. "Factors Affecting Wound Healing", Received 2008 Nov 13; Revised 2009 Sep 29; Accepted 2009 Oct 30. J Dent Res. 2010 Mar; 89(3): 219–229.,doi: 10.1177/0022034509359125, PMCID: PMC2903966,PMID: 20139336.

12. Aiko K., Natsume S., Shinichi H., Hitoshi Y. and Koji T., "Wound Healing in Mammals and Amphibians: Toward Limb Regeneration in Mammals", Current Topics in Microbiology and Immunology (2013) 367: 33–49 DOI: 10.1007/82_2012_305.

13. Bailey & love's, "short practice of surgery", 26th Edition, it is government of india publication.

14. Shenoy K.R. et al, "Manipal Manual of surgery", 4th Edition, sriram Bhat M publication.ss

15. SRB's Manual of Surgery, Sriram Bhat .M Publication, 4th Edition.

16. Beard JM, Osborn j. Anorectal Abscess. Rakel RE, Rakel DP, "Textbook of family Medicine", 8th edition. Philadelphia publication, Saunders; 2011.

17. Laura V, Anne D, Sophie J, "Wound Healing, Cellular Regeneration and Plasticity: The Elegans Way", 2018;62(6-7-8):pg-491-505.,doi: 10.1387/ijdb.180123sj, PMID: 29938761,PMCID: PMC6161810.

18. Thomas A. Wynn, "Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases", Volume 117 , Journal of Clinical Investigation 117(3):524-9 · April 2007, DOI: 10.1172/JCI31487 · Source: PubMed.

19. Mariana T.C., Alexandra P. M., and Rui Luı´s Reis, "Using Stem Cells in Skin Regeneration: Possibilities and Reality", STEM CELLS AND DEVELOPMENT Volume 00, Number 00, 2012 Mary Ann Liebert, Inc. DOI: 10.1089/scd.2011.0539.

20. Sabine A. Emin, Martin P, and Marjana T.C, "Wound repair and regeneration: Mechanisms, signaling, and translation", Sci Transl Med. Author manuscript; available in PMC 2016 Aug 4. Published in final edited form as: Sci Transl Med 2014 Dec 3; 6(265): 265sr6.doi: 10.1126/scitranslmed.3009337, PMCID: PMC4973620, PMID: 25473038.

21. Daniel C., Ovidiu B., and Elena R., "Calendula officinalis: Potential Roles in Cancer Treatment and Palliative Care", Integrative Cancer Therapies 1–11 © The Author(s) 2018 Article reuse guidelines: sagepub.com/journals-permissions DOI: 10.1177/1534735418803766 journals.sagepub.com/home/ict.

22. Muley BP, Khadabadi SS and Banarase NB, "Phytochemical Constituents and Pharmacological Activities of Calendula officinalis Linn (Asteraceae): A Review", Tropical
Journal of Pharmaceutical Research, October 2009; 8 (5): 455-465, © Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

23. Singh M.K., Sahu P, Nagori K, Dewangan D, Kumar T. Alexander A, Badwaik H and Tripathi D,K, , "Organoleptic properties in-vitro and in-vivo pharmacological activities of Calendula officinalis Linn: An over review", J. Chem. Pharm. Res., 2011, 3(4):655-663, ISSN No: 0975-7384 CODEN (USA): JCPRC5.

24. Baskaran K, "Pharmacological Activities of Calendula Officinalis", International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064 Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391.

25. Hand book of Excipient page no. 101,104,132-135,214-215, 430-433, 188-190, 211-212, 641-642

26. MKeane F., Munn S E, A W P du Vivier, Taylor N.F, Higgins E.M., "Analysis of Chinese herbal creams prescribed for dermatological conditions", BMJ 1999; 318 doi: https://doi.org/10.1136/bmj.318.7183.563 (Published 27 February 1999)

27. Saller R, Buechi S, Meyrat R, Schmidhauser C, "Combined Herbal Preparation for Topical Treatment of Herpes labialis", Forsch Komplementärmed Klass Naturheilkd 2001;8:373–382 (doi:10.1159/000057255).

28. Hugh A. Gemmell, DC, Bert H. Jacobson, and Brad M. Hayes, "Effect of a topical herbal cream on osteoarthritis of the hand and knee: a pilot study", Journal of Manipulative and Physiological Therapeutics Effect of Herbal Cream June 2003, Copyright © 2003 by National University of Health Sciences. 0161-4754/2003/$30.00 0 doi:10.1016/S0161-4754(03)00009-5.

29. Ramsay H. M, Goddard W, Gill S, Moss C, "Herbal creams used for atopic eczema in Birmingham, UK illegally contain potent corticosteroids", Accepted 10 April 2003 ;88:1056–1057.

30. McKay, Lawrence MB, Gemmell, Hugh DC, Jacobson, Bert Hayes, "Effect of a Topical Herbal Cream on the Pain and Stiffness of Osteoarthritis: A Randomized Double-Blind, Placebo-Controlled Clinical Trial", JCR: Journal of Clinical Rheumatology: June 2003 - Volume 9 - Issue 3 - p 164-169 doi: 10.1097/01.RHU.0000073450.85179.55.

31. Kucera, M., Barna, M., Horacek, O. et al, " Efficacy and safety of topically applied Symphytum herb extract cream in the treatment of ankle distortion", Results of a randomized controlled clinical double-blind study. Wien Med Wochenschr 154, 498–507 (2004).doi.org/10.1007/s10354-004-0114-8.

32. Kucera, M., Barna, M., Horácek, O. et al. TopicalSymphytum herb concentrate cream against myalgia: A randomized controlled double-blind clinical study. Adv Therapy 22, 681–692 (2005).doi.org/10.1007/BF02849961.

33. Ashawat M.S., Saraf S. and Saraf, "Biochemical and Histopathological Studies of Herbal Cream against UV Radiation Induced Damage", Trends in Medical Research, 2: 135-141.doi: 10.3923/tmr.2007.135.141.

34. Kamkaen N., Phuntuwate W., Samee W, Boonrod A and Treesak C, "TheInvestigationof the Rabbit and Human Skin Irritation of Herbal Anti-wrinkle Cream", Thai Pharmaceutical and Health Science Journal, Vol. 2 No. 1, Jan. – Apr. 2007.

35. Ahshawat M.S., Saraf S, Saraf S, "Preparation and characterization of herbal creams for improvement of skin viscoelastic properties", First published:30 April 2008 doi.org/10.1111/j.1468-2494.2008.00442.

36. Daryabeigi R, Mohammad H., Hosseini S.A, and Mahmoud O, "Comparison of healing time of the 2nd degree burn wounds with two dressing methods of fundermol herbal ointment and 1% silver sulfadiazine cream", . 2010 Summer; 15(3): 97–101, PMCID: PMC3093171, PMID: 21589770.

37. Luanda M.A.S., Michielin M.Z., LeandroDanielski, Sandra R.S.Ferreira, "Experimental data and modeling the supercritical fluid extraction of marigold (Calendula officinalis) oleoresin", The Journal of Supercritical Fluids, Volume 34, Issue 2, June 2005, Pages 163-170.

38. Jimenez-Medina, E., Garcia-Lora, A., Paco, L. et al. "A new extract of the plant calendula officinalis produces a dual in vitroeffect: cytotoxic anti-tumor activity and lymphocyte activation", BMC Cancer 6, 119 (2006).doi.org/10.1186/1471-2407-6-119.

39. Luanda M.A.S., Eliane M.Z.Michielin, LeandroDanielski, Sandra R.S.Ferreira, "Marigold (Calendula officinalis L.) oleoresin: Solubility in SC-CO2 and composition profile", Chemical Engineering and Processing: Process Intensification, Volume 46, Issue 2, February 2007, Pages 99-106.

40. Leach MJ et al., "Calendula officinalis and Wound Healing: A Systematic Review.", Wounds: a Compendium of Clinical Research and Practice, 01 Aug 2008, 20(8):236-243 PMID: 25941793.

41. Preethi, KorengathC. K., Ramadasan, "Hepato and reno protective action of Calendula officinalis L. flower extract", IJEB Vol.47 (03) March 2009, pg. 163-168.

42. Yris M F., Carolina D., Fabiana T.M.C.Vicentini, AuroNomizo, Raquel FernandaGerlach, Maria Jose VieiraFonseca, "Protective effect of Calendula officinalis extract against UVB-induced oxidative stress in skin: Evaluation of reduced glutathione levels and matrix metalloproteinase secretion", Journal of Ethnopharmacology, Volume 127, Issue 3, 17 February 2010, Pages 596-601.

43. Leila Maria Leal Parente, Maria Auxiliadora Andrade, Luiz Augusto Batista Brito, Veridiana Maria Brianezi Dignani de Moura, Marina Pacheco Miguel, Ruy de Souza Lino-Junior, Leonice Faustino Manrique Tresvenzol, Jose Realino de Paula, Neusa Margarida Paulo, "Angiogenic activity of Calendula officinalis flowers L. in rats", *On-line version* ISSN 1678-2674, Acta Cir. Bras. vol.26 no.1 São Paulo Jan./Feb. 2011,doi.org/10.1590/S0102-86502011000100005.

44. Efstratios Efstratiou, AbdullahI.Hussain, Poonam S.Nigam, John E.Moore, Muhammad A.Ayub, Juluri R.Rao, "Antimicrobial activity of Calendula officinalis petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens", Volume 18, Issue 3, August 2012, Pages 173-176, doi.org/10.1016/j.ctcp.2012.02.003.