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Research article

# Formulation and standardization of calendula officinalis for wound healing

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## 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 quantity 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.

**Keywords:** 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. 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 [1].

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. 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 contain 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 theformulation [2].

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. The mending of a surface injury is advanced by reaching the injury surfaces with a suspension of particles of collagen and a glycol aminoglycan that is chemotactic of fibroblasts as well as endothelial cells. 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 [3].

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 [4].

Plus, it is additionally giving the skin essential supplements. 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 [5, 6].

Calendula officinalis, the pot marigold, common marigold, ruddles, Mary's gold or Scotch marigold, is a flowering plant in the daisy family Asteraceae. It is probably native to southern Europe, though its long history of

cultivation makes its precise origin unknown, and it may possibly be of garden origin. It is also widely naturalised farther north in Europe (as far as southern England) and elsewhere in warm temperate regions of the world [7, 8].

**Description**

Calendula officinalis is a short-lived aromatic herbaceous perennial, growing to 80 cm (31 in) tall, with sparsely branched lax or erect stems. The leaves are oblong-lanceolate, 5–17 cm (2– 7 in) long, hairy on both sides, and with margins entire or occasionally waved or weakly toothed. The inflorescences are yellow, comprising a thick capitulum or flowerhead 4–7 cm (1+1⁄2–3 in) diameter surrounded by two rows of hairy bracts; in the wild plant they have a single ring of ray florets surrounding the central disc florets. The disc florets are tubular and hermaphroditic, and generally of a more intense orange-yellow colour than the female, tridentate, peripheral ray florets. The flowers may appear all year long where conditions are suitable. The fruit is a thorny curved achene and weighing on average 10.1 mg (n=50) [8, 9].

**Cultivation**

Calendula officinalis is widely cultivated and can be grown easily in sunny locations in most kinds of soils. Although perennial, it is commonly treated as an annual, particularly in colder regions where its winter survival is poor, and in hot summer locations where it also does not survive [10, 11].

**Seeds of calendula officinalis**

Calendulas are considered by many gardening experts as among the easiest and most versatile flowers to grow in a garden, especially because they tolerate most soils. In temperate climates, seeds are sown in spring for blooms that last throughout the summer and well into the fall. In areas of limited winter freezing, seeds are sown in autumn for winter color. Plants will wither in subtropical summer. Seeds will germinate freely in sunny or half-sunny locations, but plants do best if planted in sunny locations with rich, well-drained soil. Pot marigolds typically bloom quickly from seed (in under two months) in bright yellows, golds, and oranges [12, 13].

Leaves are spirally arranged, 5–18 cm (2–7 in) long, simple, and slightly hairy. The flower heads range from pastel yellow to deep orange, and are 3–7 cm (1+1⁄4–2+3⁄4 in) across, with both ray florets and disc florets. Most cultivars have a spicy aroma. It is recommended to deadhead (remove dying flower heads) the plants regularly to maintain even blossom production [14, 15].

**Phytochemistry**

A number of phytochemical studies have well reported about the presence of several classes of chemical compounds, the main ones being terpenoids, flavonoids, coumarines, quinones, volatile oil, carotenoids and amino acids in the plant [16, 17].

**Terpenoids**

Various terpenoids have been reported from the petroleum ether extract of C.officinalis flowers. They include sitosterols, stigmasterols, diesters of diols, 3- monoesters of taraxasterol, ψ- taraxasterol, lupeol , erythrodiol, brein, ursadiol, faradiol-3-O-palmitate, faradiol- 3-Omyristate,

faradiol-3-O-laurate, arnidiol-3-O-palmitate, arnidiol-3-O-myristate, arnidiol-3-O-laurate, calenduladiol-3-Opalmitate, calenduladiol-3-O-myristate, oleanolic acid saponins: calenduloside AH, oleanane triterpene glycoside: calendulaglycoside A, calendulaglycoside A6-O-n-methyl ester, calendulaglycoside A6‟‟-O-n-butyl ester, calendulaglycoside B, calendulaglycoside B 6-O- n-butyl ester, calendulaglycoside C, calendulaglycoside C 6-O-n-methyl ester, calendulaglycoside C 6- O-n-butyl ester, calenduloside F6_-O-n-butyl ester, calnduloside G6-O- n-methyl ester, glucosides of oleanolic acid (mainly found in roots of grown and senescing plants) I, II, III, VI, VII, and glucuronides (mainly found in flowers and green parts) F, D, D2, C, B and A. One new triterpenic ester of olanane series has been isolated from flowers was cornulacic acid acetate from flowers [18, 19].

**Flavonoids**

Various flavonoids have been isolated from the ethanol extract of the inflorescence of C. Officinalis. They include quercetin, isorhamnetin, isoquercetin, isorhamnetin-3-O--D-glycoside, narcissin, calendoflaside, calendoflavoside, calendoflavobioside, rutin, isoquercitrin neohesperidoside, isorhamnetin-3-Oneohesperidoside, isorhamnetin-3-O-2G- rhamnosyl rutinoside, isorhamnetin-3-Orutinoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside [20].

**Coumarins**

The ethanol extract of the inflorescence of the C. officinalis reported to contain coumarins - scopoletin, umbelliferone and esculetin [21].

**Quinones**

Quinones reported from C. officinalis were plastoquinone, phylloquinone, _-tocopherol in the chloroplast, ubiquinone, phylloquinone, _ tocopherol in mitochondria, and phylloquinone in the leaves [22].

**Volatile oil**

C. officinalis flowers contain maximum volatile oil at full flowering stage (0.97 %) and minimum during the preflowering stage (0.13 %). The composition also showed different patterns at different phases of vegetative cycles. Various monoterpenes and sesquiterpenes have been reported in the volatile oil : α-thujene, α -pienene, sabinene, β-pienene, limonene, 1,8- cineol, p-cymene, trans- β -ocimene, ϒ-terpenene, δ-3-carene, nonanal, terpene-4-ol, 3- cylohexene-1-ol, α- phellandrene, α -terpeneol, geraniol, carvacrol, bornyl acetate, sabinyl acetate, α - cubebene, α -copaene, α-bourbonene, cubebene, α-gurjunene, aromadendrene, β -caryophyllene, α-ylangene, α-humulene, epibicyclo-sequiphellandrene, germacrene D, alloaromadendrene, β -saliene, calarene, muurolene, δ -cadinene, cadina 1,4-diene, α-cadinene, nerolidol, palustron, endobourbonene, oplopenone, α-cadinol, Tmuurolol. The essential oil was found to be rich in α -cadinene, α -cadinol, t-muurolol, limonene, and 1,8-cineol with p-cymene at lower levels at the post-flowering periods [23].

**Carotenoids**

The methanol extract of leaves, petals and pollens of C. officinalis flowers showed a number of carotenoids. The carotenoids found in the pollens and petals were neoxanthin, 9Z-neoxanthin, violaxanthin, luteoxanthin, auroxanthin, 9Z-violaxanthin, flavoxanthin, mutatoxanthin, 9Zanthroxanthin, lutein, 9/9‟A-lutein, 13/13‟Zlutein, α -cryptoxanthin, β-cryptoxanthin, z- cryptoxanthin, lycopene, α -carotene, and β- carotene. Total carotenoids (mg/g dry weight) was 7.71 % for petals and1.61 % for pollens [24].

Reported carotenoid compositions of the leaves and stems reported were neoxanthin, 9Z- neoxanthin, violaxanthin, luteoxanthin, 9Zviolaxanthin, 13Z-violaxanthin, antheraxanthin, mutatoxanthin epimer 1, mutatoxanthin epimer 2, lutein, 9/9‟ 2-lutein,α-cryptoxanthin, β- cryptoxanthin, β-carotene. Total carotenoids (mg/g dry weight) for the leaves is 0.85 % and for stems 0.18 %. Glycosides of quercetin and isorhamnetin were the predominant components of the flavonoids, while betacarotene and lutein were the most abundant carotenoids [25].

**Amino acids**

The ethanol extract of the flowers of the plant is reported to show the presence of 15 amino acids in free form: alanine, arginine, aspartic acid, aspargine, valine, histidine, glutamic acid, leucine, lysine, proline, serine, tyrosine, threonine, methionine and phenylalanine.

Amino acid content of the leaves is about 5 %, stems 3.5 % and flowers 4.5 %.

**Carbohydrates**

The ethanol extract of the inflorescence of plant showed the presence of polysaccharides, PS-I,- II, and -III having a (1_3)-_-D-galactam backbone with short side chains at C-6 comprising _- araban- (1_3)-araban and alpha-L-rhamnan-(1_3)- araban along with monosaccharides [26].

**Lipids**

The lipids in the petroleum ether extract of the seeds, leaves and flowers of C. officinalis have been analyzed. The amount of neutral lipids in the seeds was 15.7 %, phospholipids 0.6 % and glycolipids 0.9 %. Fatty acids of monols, sterol esters, 3-monoesters, 3-monoester diols reported in flowers were lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acid. The fatty acids of marigold seeds contain about 59% of an 18:3 conjugated trienic (trans-8,trans-10, cis- 12) acid and about 5% of 9-hydroxy-18:2 (trans-9,cis-11) acid - dimorphecolic acid [43,44] one oxygenated fatty acid also reported from the seed oil of C. officinalis was D-(+)-9-hydroxy- 10,12-octadecadienoic acid [27].

**Other constituents**

Other phtytochemicals include the bitter constituent, loliolide (calendin), calendulin and n- paraffins.

**Pharmacological activities**

**Phagocytosis**

Three polysaccharides isolated from an aqueous extract of Flos Calendula enhanced phagocytosis in human granulocytes in vitro in the colloidal carbon clearance test. Intraperitoneal injection of a View metadata, citation and similar papers at core.ac.uk brought to you by CORE provided by Open SIUC polysaccharide fraction isolated from an aqueous extract of the flowers to mice (10 mg/kg body weight) enhanced phagocytosis. Intraperitoneal administration of an unsaponifiable fraction (0.5 ml) of a hydroalcoholic extract of the flowers weakly stimulated

phagocytosis in mice inoculated with Escherichia coli. However, the hydroalcoholic extract was not active.

Antimicrobial activity [28].

The essential oil of the flowers inhibited the growth in vitro of Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albican6. A flavonoid fraction isolated from the flowers inhibited the growth in vitro of S. aureus, Sarcina lutea, E. coli, Klebsiella pneumoniae and Candida monosa. However, chloroform, ethanol, methanol or water extracts of the flowers did not inhibit bacterial growth in vitro. Acetone, ethanol or water extracts inhibited the growth in vitro of the fungus Neurospora crassa. Extracts of the flowers inhibited the growth in vitro of Trichomonas vaginalis. Oxygenated terpenes appear to be responsible for the antimicrobial activity [29].

**Antiviral activity**

A tincture of the flowers suppressed the replication of herpes simplex, influenza A2 and influenza APR-8 viruses in vitro14. However, an aqueous extract of the flowers was not active. A chloroform extract of the flowers inhibited the replication of HIV-1 in acutely infected lymphocytic MOLT-4 cells in vitro (IC50 0.4 mg/ml) 15. A chloroform extract also inhibited HIV-1 reverse transcriptase activity in a dose-dependent manner (ED50 51.0 mg/ml). A 5% hot aqueous extract of the flowers (2 ml) inhibited the replication of encephalitis virus after intraperitoneal administration to mice.

**Wound-healing activity**

External application of a hydroalcoholic extract accelerated the rate of contraction and epithelialization of excision wounds in rats. A 3% freeze-dried aqueous extractof the flowers induced vascularization in the chick chorioallantoic membrane assay. Histological sections of the treated chorioallantoic membranes also indicated the presence of hyaluronan, a tissue glycosaminoglycan associated with neovascularization.

Clinical pharmacology:

Although no randomized, controlled clinical trials have been performed, two case reports in the early medical literature support the traditional use of Flos Calendula. The reports describe the use of a strong tincture of the flowers applied on compresses to reduce inflammation and suppuration, and to accelerate the healing of wounds [30].

## MATERIAL AND METHOD

*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.

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.

**Figure 1:** Tincture prepared by maceration process Represented

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 [31].

**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.

**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.

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.

**Procedure**

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. • Then the borax water should add drop by drop with vigorous stirring into the oily portion. • Then melted mass should be allow to cool to get desire consistency.

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. • Then the perfume and rose oil also added in the formulation as flavoring and fragrance properties.

**Table 1:** Cream Formulation

| | FORMULA % W/W | | |
|---|---|---|---|
| **Ingredients** | **A** | **B** | **C** |
| Stearyl Alcohol | 3.5 | 3.5 | 3.5 |
| Borax | 0.05 | 0.05 | 0.05 |
| Beeswax | 5 | 5 | 5 |
| Rose Oil | 0.8 | 0.8 | 0.8 |
| Oilve Oil | 11.6 | 11.6 | 11.6 |
| Polysorbate 80 | 40.7<br>5 | 40.7<br>5 | 40.<br>75 |
| Methyl Paraben | 0.1 | 0.1 | 0.1 |
| Propyl Paraben | 0.2 | 0.2 | 0.2 |
| Ascorbic Acid | 4 | 4 | 4 |
| Calendula Extract | 20 | 17 | 14 |
| Water | Q. S up to 50gm | Q. S up to 50gm | Q. S Up to 50gm |

## RESULT

**Table 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 3:** TLC data ratio 2:1:1

| Sample No. | Rfa | Rfb | Rfc | Average |
|---|---|---|---|---|
| Sample 1 | 0.85 | 0.72 | 0.87 | 0.81 |
| Sample 2 | 0.71 | 0.66 | 0.77 | 0.71 |
| Sample 3 | 0.66 | 0.76 | 0.85 | 0.75 |

**Table 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** |

**Figure2:** Identification of drug by TLC observed sample Peaks for ratio 1:1:1 Represented

**Figure3:** Identification of drug by TLC observed sample Peaks for ratio 2:1:1 Represented

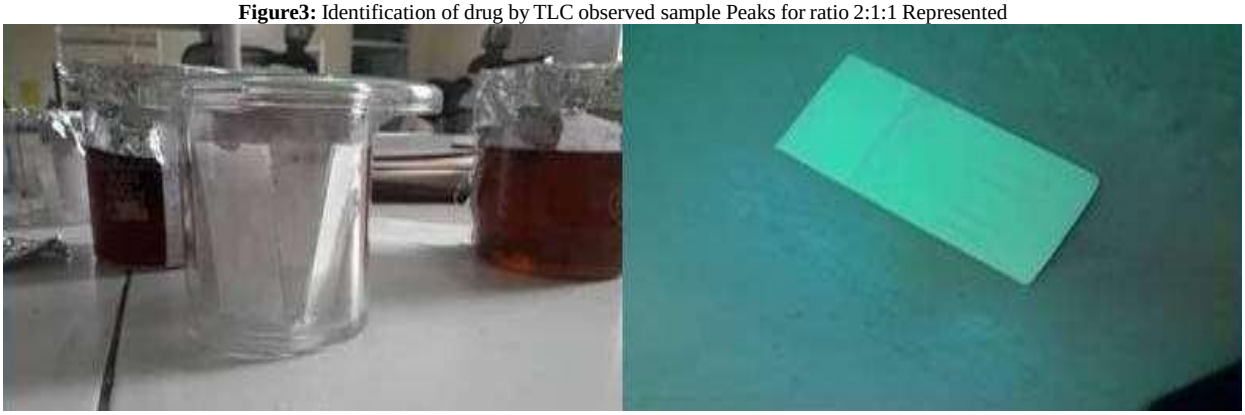
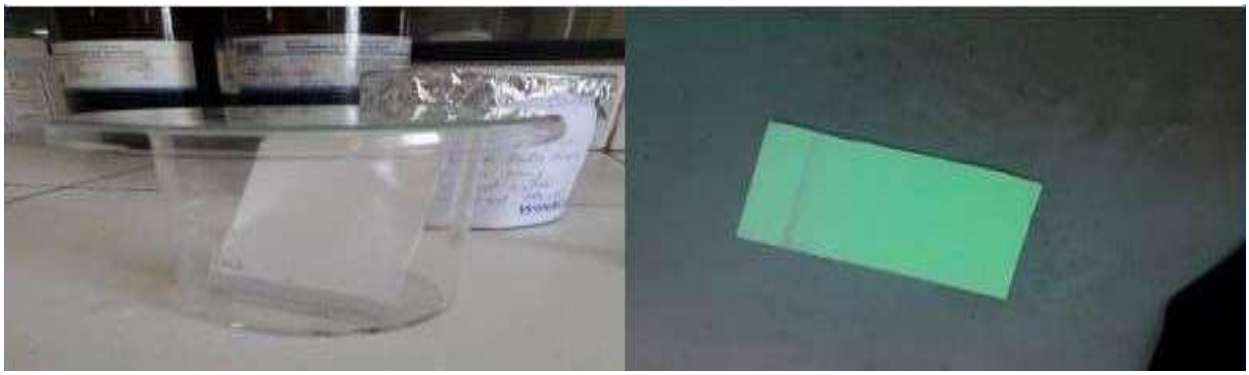


**Figure4:** Identification of drug by TLC observed sample Peaks for ratio 1:2:1Represented



**Evaluation parameters**

**PH Test**

The pH of the cream was determined using a pH meter 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.

**Table-5:** pH Evaluation parameter of all formulation

| Evaluation Parameters | Acceptance Criteria | A | B | C |
|---|---|---|---|---|
| Ph | 4.5-6 | 6.1 | 5.7 | 5.6 |

**Spreadability Test**

Utilizing a Spreadability device determined the product's Spreadability. Two glass slide (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 gram weight was allowed to rest on the upper slide for a period of one to two minutes. After the weight was removed, a force was applied to the top slide over the pulley to expose it to the force. Theamount 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 (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-6:** Spreadability Evaluation parameter of all formulation

| Evaluation Parameters | Acceptance Criteria | A | B | C |
|---|---|---|---|---|
| Spreadability | 8.50-11.50 | 5.5 | 10.8 | 10.8 |

**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.

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.

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.

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

| Sample | Quantity of Sample | Zone of inhibition Diameter (cm) |
|---|---|---|
| Sample A | 0.30 gm | 1.0 |
| Sample B | 0.30 gm | 1.4 |
| 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 to20 rpm.

The viscosity of the home-grown cream result was appeared in the scope of 500-1000 cps which ensures 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.

**Homogeneity 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.

**Marketed product of calendula officinalis**

**Bio India calendula officinalis ointment**

Bio India Calendula Officinalis Ointment is a multi-purpose ointment used for its antiseptic and healing properties. It is used for various reasons like relieving menstrual cramp pains, reducing swelling and inflammation on the skin and promoting wound healing.

Marketed By: Bio-India Pharma Pvt. Ltd.


**Calendula officinalis Q**

Mother Tincture -Promotes the Healing of Injured Skin, crevices, abrasions, chapped Skin, and Insect Bites

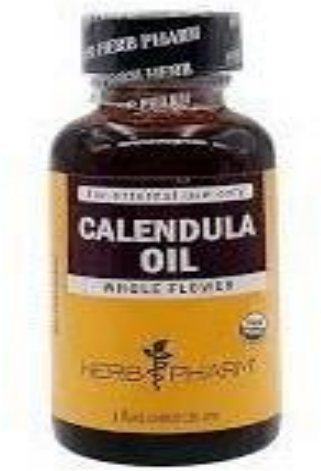
Marketed By: SBL PVT.LTD


**Calendula officinalis mother tincture Q**

It is a multipurpose homoeopathy medicine used for a number of purposes. It is used to help in treating cuts and wounds. It works by soothing the bleeding after injuries and

hence is very well useful in treatment for keloids. These are very efficient in treating any type of ulcer. Rheumatic pains are also addressed with this remedy.

Marketed By: LDD Bioscience Pvt. Ltd




**Dabur calendula soap**

Dabur Calendula Soap is an antioxidant soap that protects from UV-B radiation. It is formulated with natural calendula oil, honey, and kesar that helps heal wounds, prevents scars, improves the appearance of the skin and protects against fungal infections. Kesar helps to improve skin complexion and reduce acne and wounds.

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## 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 [32].

The studies of mechanism of action of wound healing capacity of C.officinalis have shown a slight better edgeof 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 [33].

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 [34].

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 variousscars, 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 fails. Therefore order to reduce modify the leads structure. Which is important for the invitro study. Swiss ADME software helps to computation of the key such as physicochemical pharmacokinetics drug like and other multiple parameters studies involve it.Pubchem is the another web tool which also gives the information related to molecules or lead compound.which can determine the structure name of the chemical compound ,toxicity, spectral information etc.

This is concluded that the lead compound analysis done by this two tools which is pubchem and Swiss ADME.

**Review of literature**

The research paper titled "THE PHARMA INNOVATION: Recent Advances in Novel Topical Drug Delivery System" by Debjit B., Harish G., B. Pragati K, S. Duraivel, and K.P. Sampath Kumar, published in 2012, explores recent advancements in topical drug delivery systems. The literature review likely covers various innovative techniques, formulations, and technologies employed in delivering drugs through the skin. This may include transdermal patches, microemulsions, nanocarriers, and other cutting-edge methods aimed at enhancing drug permeation, efficacy, and patient compliance. The paper likely offers insights into the challenges, opportunities, and future directions in the field of topical drug delivery.

The research paper titled "Wound Healing - A Literature Review" by Ana Cristina de Oliveira Gonzalez, Tila Fortuna Costa, Zilton de Araújo Andrade, and Alena Ribeiro Alves Peixoto Medrado, published in the Brazilian Journal of Dermatology in 2016, offers a comprehensive overview of the wound healing process. The literature review likely covers various aspects of wound healing, including the physiological mechanisms involved, stages of wound repair, factors influencing healing outcomes, and current therapeutic interventions. It may also discuss recent advancements in wound care, emerging therapies, and challenges in managing different types of wounds. Overall, the paper provides valuable insights into the complex process of wound healing, aiming to enhance understanding and improve clinical management strategies.

The patent titled "Compositions, Articles, and Methods for Improving Wound Healing" by Michaeli D, Francisco S, Calif, Horne T.R., and Thurman K., filed on August 15, 1986, and assigned Patent Number 4,837,024, likely describes novel compositions, articles, and methods aimed at enhancing the wound healing process. The patent may detail specific formulations, materials, or techniques designed to promote tissue repair, reduce inflammation, prevent infection, or accelerate wound closure. It could cover a range of applications, including pharmaceuticals, medical

devices, dressings, or topical treatments, offering innovative solutions for improving wound care outcomes.

The research paper titled "Formulation and Evaluation of Multipurpose Herbal Cream" by Dhyani A, Chander V, and Singh N, published in the Journal of Drug Delivery and Therapeutics in 2019, likely presents a study on the development and assessment of a versatile herbal cream. The paper may describe the formulation process, which likely involves combining various herbal ingredients known for their therapeutic properties. Additionally, the evaluation likely includes tests for stability, efficacy, safety, and sensory characteristics of the herbal cream. Overall, the paper aims to contribute to the field of herbal medicine and skincare by offering a novel multipurpose cream formulation with potential benefits for various dermatological conditions.

The article titled "World Journal of Pharmaceutical Research", Volume 7, Issue 7, 2018, Pages 573-591, authored by Saudagar R. B. and Sisodiya M. H., likely covers a range of pharmaceutical research topics. Without specific information on the content of the article, it could include studies on drug development, formulation, delivery systems, pharmacology, pharmacokinetics, or clinical trials. The journal may feature original research, reviews, or case studies aimed at advancing pharmaceutical science and promoting innovation in drug discovery and development.

The article titled "Preparation and Evaluation of Herbal Cosmetic Cream" by Singh M, Sharma S, Khokra S.L., Sahu R.K., and Jangde R, published in Pharmacology online in 2011, likely presents a study on the formulation and assessment of a herbal cosmetic cream. The paper likely describes the process of preparing the cream using herbal ingredients known for their cosmetic benefits. Additionally, it may include evaluations of the cream's stability, safety, efficacy, and sensory properties. Overall, the article contributes to the field of herbal cosmetics by offering insights into the development of natural and potentially beneficial skincare products

The article titled "Labeling of Cosmetic Products" by Nicola L. and Luigi R., published in Cosmetics in 2018, provides insights into the regulatory requirements and best practices for labeling cosmetic products. It likely covers various aspects such as ingredient listing, product claims, usage instructions, warnings, and regulatory compliance. The paper may discuss the importance of accurate and transparent labeling for consumer safety, as well as strategies for ensuring compliance with national and international regulations. Overall, it serves as a valuable resource for manufacturers, regulators, and consumers seeking information on cosmetic product labeling standards and guidelines.

The article titled "Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources" by Pan Y., Gerhard L., Gao S., Zhou S., Zhi-Ling Yu, Chen H., Zhang S., Tang M., Jian-Ning Sun, and Kam-Ming Ko, published in Evidence-Based Complementary and Alternative Medicine by Hindawi Publishing Corporation in 2014, likely offers an in-depth exploration of traditional indigenous medical practices and the resurgence of interest in herbal resources. The paper probably provides a historical overview of traditional medicine, highlighting its cultural significance and role in healthcare. It may also discuss contemporary efforts to preserve, study, and integrate traditional healing practices into modern healthcare systems. Overall, the article likely contributes to the understanding of traditional medicine's value and its potential in addressing current health challenges.

Bailey & Love's "Short Practice of Surgery" is a renowned surgical textbook, highly regarded for its comprehensive coverage of surgical principles, techniques, and procedures. The 26th edition, published as a government of India publication, likely continues the tradition of providing updated and authoritative information on various surgical specialties. It serves as an essential reference for medical students, residents, and practicing surgeons, offering insights into the latest advancements in the field of surgery.
The "Manipal Manual of Surgery," authored by Shenoy K.R. et al., is a comprehensive textbook that serves as a valuable resource for medical students and surgical practitioners. The 4th edition, published by Sriram Bhat M Publication, likely continues to provide detailed coverage of surgical principles, procedures, and techniques relevant to clinical practice. It may include updated information, illustrations, and clinical insights to aid in understanding surgical concepts and decision-making. Overall, the book is likely to be a reliable guide for students and professionals navigating the field of surgery.

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