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Formulation and Evaluation of a Polyherbal Gel for Skin Disorders

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ABSTRACT:
The aim of the present investigation was to formulate and evaluate the herbal gel containing Marigold, Garlic, Honey, Cocoa Powder Aloe Vera for wound healing and antimicrobial potential. Marigold, Garlic, Honey, Cocoa Powder Aloe Vera used in the preparation possess an excellent anti-inflammatory, antioxidant and healing potential. Gelling agent used in this study was Carbopol 934P. In the present study five formulations containing different concentration of carbopol and honey were formulated and optimized. The optimized formulations were selected for further studies. Optimized gel was then loaded with active drug. The formulations were characterized for initial physicochemical parameters i.e. surface pH, spreadability, viscosity and antimicrobial susceptibility test to observe toxicity or side effects. The result revealed that the surface pH was within the range of skin pH. The viscosity and spreadability of the gel was appropriate and zone of inhibition was also satisfactory for optimized formulations G4 and G5. The preparations were stable under normal storage conditions and did not produce any skin irritation, i.e., erythema and oedema for about a month, when applied over the skin.

KEYWORDS: Marigold, Honey, Garlic, Aloe vera

INTRODUCTION:

Wound, an incident often experienced by humans in daily activities, is simply defined as the broken of skin tissue. Many factors can cause the damage of skin tissue, such as physical friction or high temperature that disrupts the arrangement of epithelial or mucosa cells on the skin. The wound healing process is an essential process with which the skin's function as the body protection means can be restored immediately. The wound healing process consists of several stages started from inflammation, a proliferation that includes epithelialization, angiogenesis, and skin remodeling. During those complex processes, it is necessary to maintain the wound's sterility, protect the wound from contamination that can cause infection, prevent dehydration (moisture is needed for the new tissue arrangement), and also absorb or eliminate the wound exudate.

According to the Food and Drug Administration (FDA), preparations for wound healing, or also known as wound dressing combined with drugs, are divided into three categories: the wound dressing in solid forms, preparation of semisolid (ointments, creams, gels), and wound cleaning fluid. The application of appropriate dosage forms and formulations in the wound therapeutic is required to accelerate the wound

healing process. One of the suitable dosage forms for this purpose is a gel. A gel is a rigid structure formed by a cross-linking network of three dimensions that swells in an appropriate solvent; when the solvent is water, it is known as hydrogel. The high-water content in the gel formula is suitable for the environment needed for wound healing. It creates a moist environment and absorbs wound exudates to accelerate the wound healing process. Besides, the gel can provide a cool sensation when applied to the injured skin so that it can reduce the pain experienced by the patient. Further, it is also easily applied and removed from the skin.

There are so many topical wound healing products on the market. Most of them contain active pharmaceutical ingredients that belong to antiseptics and antimicrobials categories, such as Neomycin, Mupirocin, Povidone Iodine, Hydrogen Peroxide, and Bacitracin. The use of antimicrobials in the medical world lately starts to cause debates due to the significant risk of the bacteria resistance event. It triggers many kinds of research that focus on searching for new drug materials that can be used as wound healing agents. One section of the searches involves the use of natural wealth. Spread in nature, many plants have wound healing activity. One of the plants is the Yellow Marigold (Tagetes erecta), or also known as African marigold. Tagetes erecta leaf is traditionally used to heal wounds by being chewed or chopped, then placed on the injured skin until it is healed. Tagetes erecta leaf has some pharmacology effects, such as an anti-inflammatory and antibacterial activity that relates to the wound healing process . Several studies have proven the effectiveness of this plant in the wound healing process. The ethanol extract of Tagetes erecta leaves has shown its ability to accelerate wound closure.

In the pharmaceutical formulation design, the compatibility between the active ingredients and other excipients in the dosage form should be considered. Ethanolic extract has good compatibility with water; so in this research, the ethanolic extract of Tagetes erecta leaf is formulated as hydrogel. The urgency of this research is to find a natural ingredient that can be used as wound healing medicine and to formulate the ingredient into the dosage form that fulfills the pharmaceutical aspects, such as efficacy, safety, and quality. In this study, the formulations of wound healing gel preparation using Tagetes erecta leaves extract with various concentrations of gelling agent (Carbopol 934) and humectant (Honey) were done. The evaluation was carried out on the physical properties of the gel, the stability of the gel, and the wound healing activity of each formula, in order to study the characteristics of the gel-based on the formulation consideration.

APPLICATION OF GEL:

- AVOID ORAL DRUG DEGRADATION
- EXTEND THE PRODUCT FOR ECONOMICAL REASONS E.G. PAINT
- USED IN GEL FILTRATION
- AEROGELS
- GLYCOGELATIN GELS ARE USED AS A BASIS FOR MEDICATED PESTILLES.
- FORMULATION OF SOME SUPPOSITORIES E.G. GLYCERINSUPPOSITORIES B.P.
- USED IN HARD AND SOFT GEL CAPSULES.

Topical Drug Delivery System:

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentrations. The route of administration has a significant impact on the therapeutic outcome of a drug. Skin is one of the most readily accessible

organs on human body for topical administration and is main route of topical drug delivery system. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominates the system for topical delivery, but foams, spray, medicated powders, solutions, as well as medicated adhesive systems are also in use.

- External topical that are spread, sprayed, or otherwise dispersed on to cutaneous tissues to cover the affected area
- Internal topical that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity.

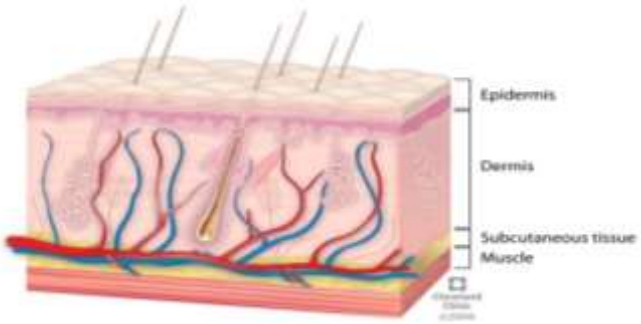
Advantages of Topical Drug Delivery System:

- Avoidance of first pass metabolism.
- Convenient and easy to apply.
- Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes,
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input.
- Avoids fluctuation in drug levels, inter- and intrapatient variations.
- Ability to easily terminate the medications, when needed.
- A relatively large area of application in comparison with buccal or nasal cavity
- Ability to deliver drug more selectively to a specific site.
- Providing utilization of drugs with short biological half-life,
- Improving physiological and pharmacological response.
- Improve patient compliance.
- Provide suitability for self-medication.

Disadvantages of Topical Drug Delivery System:

- Skin irritation of contact dermatitis may occur due to the drug and/or excipients.
- Poor permeability of some drugs through the skin.
- Possibility of allergenic reactions.
- Can be used only for drugs which require very small plasma concentration for action
- Enzyme in epidermis may denature the drugs
- Drugs of larger particle size not easy to absorb through the skin**.**

SKIN:



The human skin plays a significant role in physiological functions of sensation, protection, thermoregulation, defense system, and metabolic mechanisms to help in maintaining homeostasis . It is mostly exposed to different environmental factors such as harmful radiation, toxic chemicals, and pathogens. According to Ayurveda, the skin is one of the essential sense organs. Ayurveda has its own unique principles of diagnosis and treatment of various skin diseases under the heading of Kuṣṭha. The ultraviolet (UV) portion of sunlight is responsible for various skin disorders. Continuous exposure to UVB leads to various adverse effects on the skin. The increase in awareness about the photoaging and carcinogenic effects of UV radiation (UVR) resulted in tremendous increase in the demand for herbal skincare products. The most frequently used inorganic filters in the market possess titanium dioxide (TiO2) and zinc oxide (ZnO). These UV filters are suggested to be the most frequent cause of dermal toxicity and contact allergy, which is caused by a wide range of chemicals present in sunscreens. The herbal products exhibit various therapeutic properties and have always been used for centuries in the treatment of many skin disorders. Several herbal products have been uncovered for their therapeutic potential and are gaining considerable attention in the market as skincare products. Internationally, numerous studies have been conducted on many herbal products to reveal their therapeutic potential via various in vivo and in vitro models. However, future long-term studies and new approaches are required in this area Human skin is composed of three distinct layers: epidermis, dermis and hypodermis, with varying degrees of specialization within each layer.The epidermis and dermis are well characterized, but very little attention has been given to the hypodermis and retinacula.

Three layers of tissue make up the skin:

- **Epidermis**, the top layer.
- **Dermis**, the middle layer.
- **Hypodermis**, the bottom or fatty layer.

WOUND HEALING:

WOUND:

- It is a injury caused by external force and it can involve any tissue or organ.
- A wound is a break in the integrity of the skin or tissues often which may be associated with disruption of the structure and function

CLASSIFICATION OF WOUND:

1. Clean Wound
2. Colonized Wound
3. Contaminated Wound
4. Infected Wound

HEALING:

- tissue Healing is the body's response to injury in an attempt to restore normal structure and function.
- Response of an organism to a physical disruption of a tissue/organ with an aim to repair or reconstitute the defect and to re-establish homeostasis.
- During healing, a complex cascade of cellular events occur to achieve resurfacing, reconstitution and restoration of tensile strength of injured.

The progress of healing involves two distinct processes:

- REGENERATION
- REPAIR

PHASES OF WOUND HEALING:

1.Inflammatory phase: It can be broken down into further

a) Clot formation

b) Early inflammation

c) Late inflammation

2.Proliferative phase

3. Maturation phase

1. INFLAMMATORY PHASE:

a) Clot formation:

- Blood vessel contraction initiated by platelet degranulation of serotonin, which acts on endothelial cell and increases the permeability of the vessel, allowing a protein rich exudate to enter the wound site
- A platelet plug formation
- Activation of extrinsic and intrinsic clotting mechanism.

2. PROLIFERATIVE PHASE:

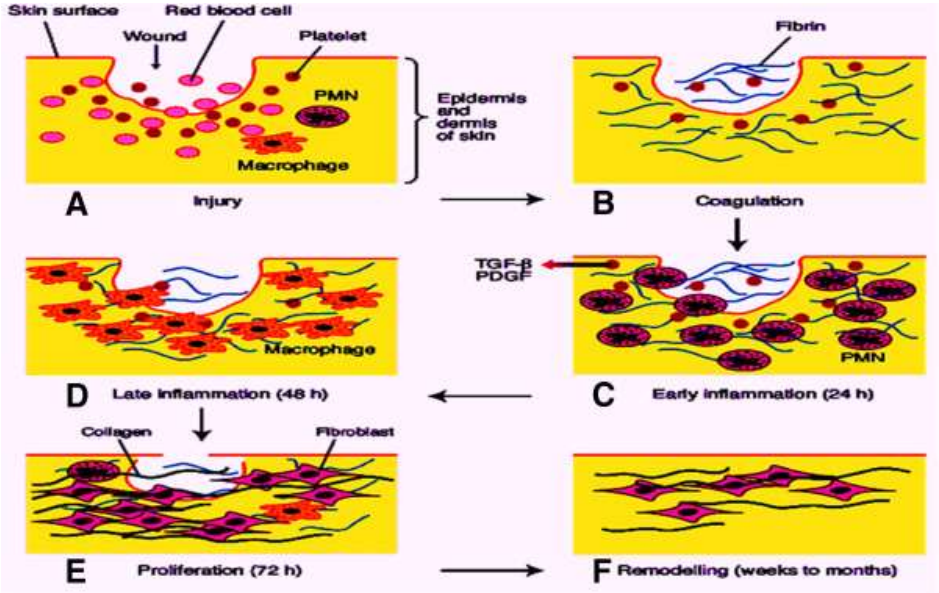
- Characterized by formation of granulation tissue in the wound.
- 2 key cell types are present in this phase:

a) fibroblasts

b) endothelial cells

3. MATURATION PHASE:

- Begins 5 to 7 days after injury.
- There is conversion of granulation tissue to fibrous connective tissue and decrease parallelism of collagen to the plane of the wound.
- Maturation of the epithelial layer quickly follows formation of the epithelial seal.



DRUG PROFILE:

A] Allium sativum:

One of the traditional medicines that can be used to treat wounds is garlic (Allium sativum) Garlic contains compounds essential chemical that is good for their body. Essential oils have antibacterial and antiseptic properties to prevent infection in the wound.

Common names: Garlic

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Liliopsida
Order:	Asparagales
Family:	Alliaceae
Subfamily:	Allioideae
Tribe:	Allieae
Genus:	Allium
Species:	A. sativum

Chemical constituents:

Garlic has a variety of bioactive compounds, including organosulfur compounds, saponins, phenolic compounds, and polysaccharides. The major active components of garlic are its organosulfur compounds, such as diallyl thiosulfonate (allicin), diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), E/Z-ajoene, S-allyl-cysteine (SAC), and S-allyl-cysteine sulfoxide (alliin). The total amount of saponin in purple garlic was almost 40 times higher than that in white garlic, and several saponin compounds were only found to exist in purple garlic, such as desgalactotigonin-rhamnose, proto-desgalactotigonin, proto-desgalactotigonin- rhamnose, voghieroside D1, sativoside B1-rhamnose, and sativoside R1. Moreover, garlic contained more than 20 phenolic compounds, with higher contents than many common vegetables. The main phenolic compound was β-resorcylic acid, followed by pyrogallol, gallic acid, rutin, protocatechuic acid, as well as quercetin. Furthermore, garlic polysaccharides were reported to contain 85% fructose, 14% glucose, and 1% galactose.

ORGANOLEPTIC PROPERTIES:

Colour: pinkish-white

Odour: odoriferous

Taste: soft, sweet buttery flavour.

Size: 1.5- 2.5 cm

Discription: A bulb of garlics, the most commonly used part of the plant, is divided into numerous fleshy sections called cloves. The cloves are used as seed, for consumption (raw or cooked), and for medicinal purposes. The leaves, stems (scape) and flowers (bulbils) on the head (spathe) are also edible and most often consumed while immature and still tender. The papery, protective layers of 'skin' over various parts of the plant and the roots attached to the bulb are the only parts not considered palatable.

Biological Source

Garlic is the ripe bulb of *Allium sativum* Linn., belonging to family Liliaceae.

Geographical Source

Garlic occurs in central Asia, southern Europe, and United States. It is widely cultivated in India.

Melting Point: 163 °C.

Storage: Store in an airtight continer, away from direct sunlight nd kept in at room temperature

Therapeutic category: herbal supplement

Mechanism of Action: Garlic has allicin active substances that have antibacterial and anti-inflammatory properties that can give effect to the wound recovery of ethanol extract of Allium sativum has a significant wound healing activity in rats. Flavonoids, saponins, alkaloids, and phenolics are known to have an active antibioticc principle. Allicin, the active component of garlic, has been shown to have antimicrobial and anti-inflammatory properties. Garlic has also been used historically by many cultures to heal wounds. Several animal studies have shown that garlic extracts increase the rate of wound healing and decrease the rate of infection.

Biological Function:

1. Antioxidant Activity
2. Anti-Inflammatory Activity
3. Antimicrobial Activity
4. Modulating Immune System
5. Cardiovascular Protection

6. Anticancer Activity

B) Aloe vera:

The Aloe vera plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. The name Aloe vera derives from the Arabic word "Alloeh" meaning "shining bitter substance," while "vera" in Latin means "true." 2000 years ago, the Greek scientists regarded Aloe vera as the universal panacea. The Egyptians called Aloe "the plant of immortality." Today, the Aloe vera plant has been used for various purposes in dermatology.

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Asphodelaceae
Subfamily:	Asphodeloideae
Tribe:	Aloeae
Genus:	Aloe

Anatomy:

The plant has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits that contain numerous seeds. Each leaf is composed of three layers: 1) An inner clear gel that contains 99% water and rest is made of glucomannans, amino acids, lipids, sterols and vitamins. 2) The middle layer of latex which is the bitter yellow sap and contains anthraquinones and glycosides. 3) The outer thick layer of 15–20 cells called as rind which has protective function and synthesizes carbohydrates and proteins. Inside the rind are vascular bundles responsible for transportation of substances such as water (xylem) and starch (phloem)

Active components:

Aloe vera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids.

Description:

Most *Aloe* species have a rosette of large, thick, fleshy leaves. *Aloe* flowers are tubular, frequently yellow, orange, pink, or red, and are borne, densely clustered and pendant, at the apex of simple or branched, leafless stems. Many species of *Aloe* appear to be stemless, with the rosette growing directly at ground level; other varieties may have a branched or unbranched stem from which the fleshy leaves spring. They vary in color from grey to bright-green and are sometimes striped or mottled. Some aloes native to South Africa are tree-like (arborescent).

Mechanism of action:

Glucomannan, a mannose-rich polysaccharide, and gibberellin, a growth hormone, interacts with growth factor receptors on the fibroblast, thereby stimulating its activity and proliferation, which in turn significantly increases collagen synthesis after topical and oral Aloe vera.[9] Aloe gel not only increased collagen content of the wound but also changed collagen composition (more type III) and increased the degree of collagen cross linking. Due to this, it accelerated wound contraction and increased the breaking strength of resulting scar tissue.[10] An increased synthesis of hyaluronic acid and dermatan sulfate in the granulation tissue of a healing wound following oral or topical treatment has been reported.

C) MARIGOLD:

Marigold is a spice native to India. Historically, marigold has been used all over India, China and Indonesia as a spice and medicinal agent. Marigold is a spice that enhances the flavour of foods and is the base of most Indian curries. Marigold is used in curries goes back more than 5000 years.

TAXONOMICAL CLASSIFICATION

Tagetes erecta is stout, branching herb, native to Mexico and other warmer parts of America and neutralized elsewhere in the tropics and subtropics including India and Bangladesh.

Kingdom:	Plantae
Order:	Asterales
Family:	Asteraceae
Subfamil :	Asteroideae
Class:	Magnoliopsida
Division:	Magnoliophyta
Genus:	Tagetes
Species:	erect

CHEMICAL CONSTITUENTS:

Phytochemical studies of its different parts have resulted in the isolation of various chemical constituents such as thiophenes, flavonoids, carotenoids and triterpenoids. The plant T. erecta has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, vinyl and ethyl gallate. Lutein is an oxycarotenoid, or xanthophyll, containing 2 cyclic end groups (one beta and one alpha-ionone ring) and the basic C-40 isoprenoid structure common to all carotenoids. It is one of the major constituents and the main pigment of Tagetes erecta (Dixit et al., 2013). The flower consists of carotenoids consisting of lutein, zeaxanthin, neoxanthin plus violaxanthin, βcarotene, lycopene, α-Cryptoxanthin, phytoene and phytofluene. Li-Wei (2011) report the results of a thorough phytochemical study on 22 compounds from the flowers of T. erecta by isolation of various fractions of the ethanol extract by silica gel column chromatography. They were β-sitosterol, daucosterol, 7β-hydroxysitosterol, erythrodiol-3- palmitate, lupeol, erythrodiol, 1-[5-(1-propyn-1-yl)- [2, 2-bithiophen]-5-yl]-ethanone, α-terthienyl, quercetagetin, quercetagetin-7-methylether, quercetagetin-7-O-glucoside, kaempferol, syringic acid, gallic acid, 3-α-galactosyl disyringic acid, 3-βgalactosyl disyringic acid, 6-ethoxy-2, 4- dimethylquinoline, oplodiol, (3S, 6R, 7E)-hydroxy4,7-megastigmadien-9-one, palmitin, ethylene glycollinoleate, and n-hexadecane. The chemical structures of lutein, quercetagetin and syringic acid.

PHARMACOLOGICAL ACTIVITIES:

Antibacterial activity
Antinociceptive and anti-inflammatory activity
Hepatoprotective activity
Anti-cancer activity
Anti-oxidant activity
Antiepileptic activity
Anti-fungal activity

Mechanism of action:

The activities of the marigold extract on the wound healing of albino Wister rats have been evaluated. Thirty-six male and female rats weighing 150-200g were randomly selected and divided into 4 groups (A, B, C, and D). The test rats were fed normal rat feed and water ad libitum in addition to oral administration of 1.0ml of the petal extract of marigold. Blood samples were obtained by cardiac puncture of the animals into EDTA bottles for analysis. The initial blood picture of the animals was taken before administration of the extracts to the test rats. Results showed that Tagetes erecta extract increased platelet count, white blood cell count ($p>0.05$) and shortened the bleeding and clotting times (Oguwike et al., 2013). To screen the wound healing activity of carbopol gels prepared from hydroalcoholic extracts of Gymnema sylvestere and Tagetes erecta in excision wound model and burn wound models in albino mice. Formulations of the extracts were done in the form of gels of carbopol individually and also in combination in equal ratio. In excision and burn wound models, the so treated animals showed a significant reduction in the period of epithelization and wound contraction and combined gel show wed accelerated wound healing activity may be because of synergism (Ibrahim et al., 2011).

D) COCOA:

Cocoa has a rich history in human use. Skin is prone to the development of several diseases, and the mechanisms in the pathogenesis of aged skin are still poorly understood. However, a growing body of evidence from clinical and bench research has begun to provide scientific validation for the use of cocoa-derived phytochemicals as an effective approach for skin protection.

Distribution and domestication

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Malvales
Family:	Malvaceae
Genus:	Theobroma
Species	T. cacao

Description:

Its leaves are alternate, entire, unlobed, 10–50 cm (4–20 in) long and 5–10 cm (2–4 in) broad. Cacao (*Theobroma cacao*) is one of 26 species belonging to the genus *Theobroma* classified under the subfamily Byttnerioideae of the mallow family Malvaceae. In 2008, researchers proposed a new classification based upon morphological, geographic, and genomic criteria: 10 groups have been named according to their geographic origin or the traditional cultivar name. These groups are: Amelonado, Criollo, Nacional, Contamana, Curaray, Cacao guiana, Iquitos, Marañ on, Nanay, and Purús.

Mechanism of action:

In recent years, there has been a growing interest, supported by a large number of experimental and epidemiological studies, for the beneficial effects of some phenolic substances, contained in commonly used spices and herbs, in preventing various age-related pathologic conditions, ranging from cancer to neurodegenerative diseases. Although the exact mechanisms by which polyphenols promote these effects remain to be elucidated, several reports have shown their ability to stimulate a general xenobiotic response in the target cells, activating multiple defense genes, activating a number of different molecular targets, impinging on several signaling pathways and showing pleiotropic activity on cells and tissues.

Cocoa polyphenols, mainly flavanols in both monomeric and oligomeric form, have been shown to act as a strong antioxidants, having the potential to inhibit lipid peroxidation and to effectively intercept and neutralize ROS. In this regard, cocoa flavanols have been demonstrated to be more potent than other food polyphenols. The tricyclic structure of the flavonoids determines their antioxidant effects; phenolic quinoid tautomerism and the delocalization of electrons over the aromatic system scavenge ROS. These aromatic rings directly neutralize free radicals and chelate metals (Fe^{2+} and Cu^{+}) that enhance ROS. Due to the good bioavailability, cocoa intake increases serum antioxidant capacity, protecting the endothelium from oxidative stress and endogenous ROS, although contemporary milk assumption decreases this ability. Beyond their ROS quencher activity, cocoa polyphenols effects have been mostly associated with cocoa's ability to interfere at a molecular level with numerous cellular antioxidant pathways. They strongly inhibit enzymes involved in ROS production. Enzymes inhibited by cocoa flavonoids include xanthene oxidase, NADPH-oxidase, tyrosine kinases and protein kinases. Furthermore, cocoa flavanols have been shown to upregulate antioxidant defenses by overexpressing highly protective inducible genes involved in the cellular stress response, such as the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway (Figure 2). Nrf2 is a conserved master regulator of cellular antioxidant responses. Nrf2 belongs to the Cap'n'Collar family leucine zipper transcription factors and regulates the expression of genes encoding anti-oxidant and detoxifying proteins, such as glutathione S-transferase (GST), glutathione synthetase (GSS), heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase. Among the genes activated by Nrf2, HO-1 has been the object of intensive studies for its potential role in protecting several tissues against cell death. Cocoa extract has been recently shown to efficiently induce HO-1 expression through the activation of Nrf2 in mice and, by this, to protect neurons against different challenges. Flavonoids have a number of other properties that may contribute to their protective and healing effects, including anti-inflammatory and antiplatelet activity, immunoregulatory properties and beneficial effects on vascular endothelium. The epicatechin content of cocoa is primarily responsible for its favorable impact on vascular endothelium, which is the result of both acute and chronic upregulation of nitric oxide (NO) production. NO synthesis is the most investigated endothelial function in relation to cocoa over the past 10 years, and many authors have reported that cocoa polyphenols significantly increase plasma concentrations of NO. The predominant mechanistic hypothesis is that cocoa components, in particular epicatechin, stimulate endothelial nitric oxide synthases (eNOS) activity, inhibit arginase and NADPH oxidase, leading to lower levels of superoxide and, hence, higher levels of NO. Although this is not the only mechanism involved, a substantial increase in NO synthesis may account for flow-mediated dilation and lower blood pressure following intervention treatments. Cocoa procyanidins are also potent inhibitors of mitogen-activated protein kinase kinase (MEK) and membrane type-1 (MT1)-matrix metalloproteinase (MMP). They subsequently inhibit the expression and activation of pro-MMP-2, as well as the invasion and migration of human vascular smooth muscle cells (VSMCs). Both of these mechanisms have a critical relevance, not only in flavanols cardioprotective effects, but also for their potential use in cancer prevention and photoprotection. Ramiro *et al.*studied the effects of a cocoa extract on the secretion and RNA expression of various proinflammatory mediators by macrophages. Of these, monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-α were significantly and dose-dependently diminished by the extract. All cocoa flavonoids tested were capable of reducing MCP-1 secretion after 6 h of LPS activation. The regulatory effects of cocoa flavanols on nuclear factor-κB (NF-κB) activation have also been studied. (−)-Epicatechin, (+)-catechin and their dimeric forms were

found to inhibit the NF-κB activation induced by phorbol esters in T-cells, with a clear reduction of NF-κB-DNA binding activity that leads to a reduction in interleukin 2 (IL-2) production. These effects were related to the direct interaction with the inhibitor of κB (IκB) to prevent its phosphorylation, thereby preventing NF-κB activation.

EXPERIMENTAL WORK:

PHARMACOGNOSTIC INVESTIGATION

A) Collection of Marigold, Garlic, Cocoa Powder, Aloe Vera Powder.

Collected all powder by shade drying

B) Organoleptic Characterization:

Colour, odour, shape, test of the powder and texture, fracture were observed

C) Physicochemical Characters

a. Determination of Ash value

1. Determination of total ash

Incinerated about 2-3 gm accurately weighed, of the ground drug in a tared silica dish at a temperature not exceeding 4500C until free from carbon, cool and weight. If a carbon free ash cannot be obtained in this way, exhaust the charved mass with hot water, collected the residue on an ashless filter paper, incinerated the residue and filter paper, added ignited at a temperature not exceeding 4500C. Calculated the % of ash with reference to the air dried drug.

2. Determination of Acid-Insoluble ash

To the crucible containing total ash, add 25 ml of dilute hydrochloric acid. Collected the insoluble matter on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Transferred in the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes and weighed without delay. Calculated the content of acid insoluble ash with reference to the air-dried drug.

b. Extractive values:

1. Alcohol soluble extractive value:

Macerated 5 gm of the air dried drug coarsely powdered drug (leaves and roots), with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and followed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvents, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 1050c to constant weight and weighed.

2. Water Soluble extractive value:

Macerated 5 gm of the air dried drug, coarsely powdered (leaves and roots), with 100 ml of Chloroform-water the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and followed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvents, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 1050C, to constant weight and weighed. Calculated the % of water-soluble extractive with reference to the air dried drug. [1,2]

c) Determination of Foreign Matter

The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below. Take a representative portion from a large container, or removed the entire contents of the packing if 100 g or less, and spread in a thin layer

in a suitable dish or tray. Examined in daylight with unaided eye. Transfer suspected particles, if any, to a petri dish, and examined with 10x lens in daylight.

d) Moisture Content

To check the water content and chemical quality of dried leave.

Moisture content (%) = $\frac{W2 - W3}{W2 - W1}(100)$

Where, W1= weight of empty porcelain dish

W2= weight of dish with sample before drying

W3 = weight of dish with sample after drying.

EXTRACTION

Preparation of Ethanolic Extract of Marigold (Leutin), Garlic (Allicin),Cocoa Powder (Flavonoid), Aloe Vera (Aloe Emodin), Honey :

- The Soxhlet extractor was used for extraction process.
- About 100 gm of powdered material was extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus.
- The extraction was continued until the solvent in the thimble became clear then few drops of solvent were collected in the test tube during the completion of the cycle and chemical test of the solvent was performed.
- After each extraction, the extract was evaporated to dryness in rotary vacuum evaporator.
- Moreover, some part of the extract was preserved for preliminary Phytochemical screening for the detection of various plant constituents and rest extract was used for formulation of Gel and Cream batches.

PRELIMINARY PHYTOCHEMICAL INVESTIGATION / IDENTIFICATION TESTS :

1. **For Marigold (Leutin):**
2. **For Honey: Fiehe's test:** gives instant red colour with resorcinol in hydrochloric acid**.**
3. **For Cocoa Powder (Flavoinds):** The stock solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube.
4. **For Aloe Vera (Aloe Emodin): Borax Test**: Take 10 ml of aloe solution and to it add 0.5 gm of borax and heat; a green coloured fluorescence is produced indicating the presence of aloe-emodin anthranol.
5. **For Garlic Allicin:** Allicin was estimated using high performance liquid chromatography.

EXPERIMENTAL DESIGN:

- **Formulation of Herbal Gel**

Preparation of herbal gel:

Selection of excipients:

All ingredients and excipients used are given in the Table

Method of preparation

- Accurately weighed 0.6gm Carbopol 934 was taken in a beaker and dispersed in 25 ml of distilled water.

- Kept the beaker aside to swell the Carbopol for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min.
- Take required quantity of Extract.
- Add 4 drops off coconut oil
- Add 1 ml of honey with constant stirring.

Table Formulation of Herbal gel

Sr. No.	Ingredients	Batches			Role
		A1	A2	A3	
1	Ethanolic extract	1 gm	1 gm	1 gm	Therapeutic agent
2	Carbopol 934	1.5 gm	1.5 gm	0.6 gm	Thickening agent
3	Honey	1 ml	1 ml	1ml	Humectant
4	Coconut Oil	4 Drops	4 Drops	4 Drops	Therapeutic agent
5	Distilled water	Upto 25ml	Upto 25ml	Upto 25ml	vehicle

EVALUATION OF GEL

Physical Evaluation

Physical parameters such as color and appearance were evaluated

pH

The pH of various gel formulations were determined by using digital pH meter. 2.5gm of gel was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH of each formulation was carried out in triplicate and the average values are represented. The pH of dispersions was measured using pH meter.

Spreadibility:

Spreadibility was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadibility was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 kg weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadibility. Spreadibility was calculated using the following formula:

$$S = M \times L / T$$

Where, S = Spreadibility,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide and

T = Time (in sec.) taken to separate the slide completely each other.

Homogenecity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregates.

Viscosity

Viscosity of herbal gel was determined by using Brookfield viscometer at 5, 10 20, 30 and 50 rpm using spindle no.64. Each reading was taken after equilibrium of the sample at the end of two minutes. The viscosity determination of samples was repeated three times.

Grittiness:

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence, obviously the gel preparation fulfills the requirement of freedom from particular matter and form grittiness as desired for any topical preparation **.**

[formulation and evaluation]

Stability Study:

Accelerated stability studies indicated that the physical appearance, rheological properties, spreadability in the prepared gel remained unchanged upon storage for 1 month.

Wound healing rate:

This parameter measures the speed at which the wound heals with the use of the gel.

Wound closure:

The wound closure parameter measures the extent to which the gel helps to close the wound.

Tissue regeneration:

This parameter evaluates the ability of the gel to promote the regeneration of new tissue in the wound area.

Anti-microbial activity:

This parameter evaluates the ability of the gel to prevent or control the growth of microorganisms in the wound area.

RESULT AND DISCUSSION:

The present work to increase stability of gel formulations with Carbopol 934. The prepared formulations were characterized for physical appearance, pH, spreadability, Viscosity, Greasiness, Homogeneity,etc.

Sample	Extraction Method	Solvent Used	Wt. of Sample	Extraction Value (%w/w)
Marigold, Garlic, Cocoa powder, Aloe Vera, Honey	Soxhlet Extraction	Ethanol	100gm	25%

Table: Extraction of Ethanolic Extract of Marigold (Leutin), Garlic (Allicin), Coca powder (Flavonoid), Aloe Vera (Aloe Emodin), Honey

Sr. No.	Parameter	Observation
1.	Total Ash Value (w/w%)	0.50%
2	Acid Insoluble Ash Value	1.3%
3	Alcohol Soluble Extractive Value	18%
4	Water Soluble Extractive Value	13.25%

5	Foreign Matter	1.8%
6	Moisture Content	23.5%

Table : Physico- Chemical Characters

Sr.No.	Drug Concentration	Absorbance
1	0	0
2	0.5	0.058
3	1	0.167
4	1.5	0.261
5	2	0.348
6	2.5	0.422
7	3	0.536
	Slope= y-0.1735x-0.1450	R^2= 0.9945

Table: Data of Calibration Curve

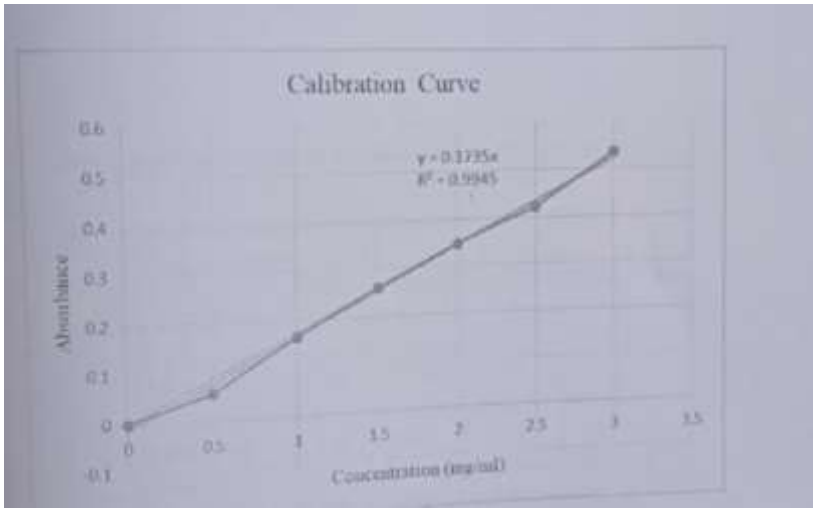


Fig: Calibration curve

Parameter	Result
Absorbance	400-500mm
Correlation Coefficient	0.9945
Slope	y-0.1735 x- 0.1450
Concentration of Allicin	0.08gm w/w
Concentration of Leutin	0.045gm w/w

Table: Result of Calibration

Sr.No.	Phytooneychemical Test	Garlic	Marigold	Aloe Vera	Cocoa	Honey
1	Alkaloids	+ve	+ve	+ve	+ve	-ve
2	Flavonoids	+ve	+ve	+ve	+ve	+ve
3	Steroids	+ve	+ve	-ve	-ve	-ve

4	Carbohydrates	-ve	+ve	+ve	+ve	+ve
5	Tanins	+ve	+ve	+ve	+ve	+ve
6	Saponins	+ve	+ve	+ve	+ve	+ve
7	Phenolic	+ve	+ve	+ve	+ve	+ve

Table: Phytochemical Analysis of Polyherbal Drug

Physicochemical Evaluation of Gel Physicochemical evaluation of Gel

1) Physical Appearance

Sr.No.	Batch	Color	Texture	Odor
1	A1	Yellowish	Smooth	Pungent
2	A2	Yellowish	Smooth	Pungent
3	A3	Yellowish	Smooth	Pungent

Table: Physical Appearance of Gel

All formulations batches were found to be Homogeneous light green gel preparations.

2) pH:

The pH values of al prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Sr. No.	Batch	pH
1	A1	6.2
2	A2	6.4
3	A3	6.5

Table pH of Formulation.

3) Homogencity:

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container.

Sr. No.	Batch	Homogencity
1	A1	Homogeneous
2	A2	Homogeneous
3	A3	Homogeneous

Table: Homogencity of Gel

4) Spreadability:

The time in seconds require to separate the two slides was taken spreadability.

Sr. No.	Batch	Spreadability (gm.sm/sec)
1	A1	15.75
2	A2	16.80
3	A3	19.78

Table : Spreadability of Formulation

5) Viscosity:

Viscosity of gel was determined by using Brookfield rotational viscometer at 5,10,20, rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times.

Sr. No.	rpm	cps
1	5	110300
2	10	112500
3	20	132700

Table: Viscosity value of herbal gel

6) Stability studies:

Accelerated stability studies indicated that the physical appearance, rheological properties, spreadability in the prepared gel remained unchanged upon storage for 1 month.

Evalution	Batches	
	Initial	After
Physical Appearance	Yellowish	Yellowish
Homogenicity	Homogeneous	Homogeneous
pH	6.2	6.5
Spreadability	15.75	19.78
Viscosity	110300	132700

Table: Stability Evaluation of Gel

The pH observed of prepared gel through 1 month storage was in between 6-7. Rheological properties and spreadability was obtained uniformly. Gel formulation was maintaining drug level after I month of accelerated stability.

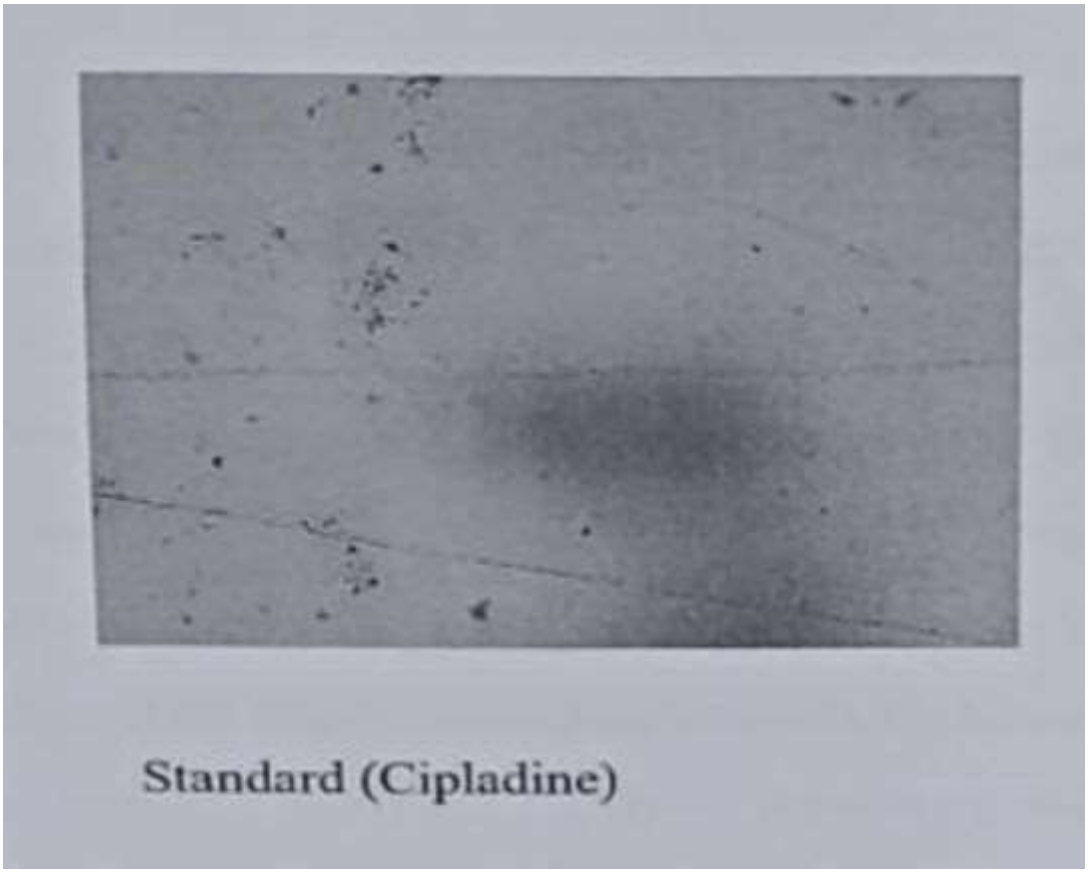
Wound closure:

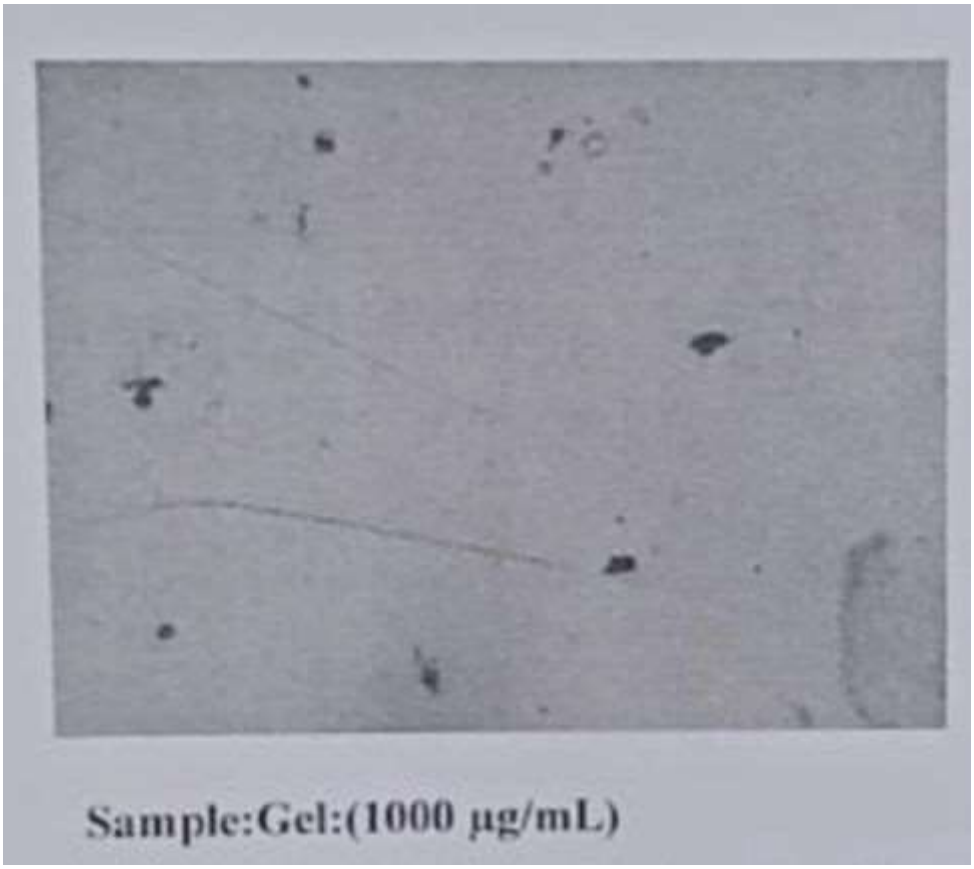
The wound healing capabilities of the Samples: gel was assayed by performing In vitro cell migration studies on L929 cells by a previously described method. Briefly, 2 x 105 cells/ml, were seeded in 6-well plates and were cultured overnight. Cells were then washed with Delbucco's Phosphate Buffered Saline (DPBS) and a scratch was made with a sterile 200µL tip. The detached cells and other cellular debris were removed by washing the cells with DPBS. The cells were treated with 1000 µg/mL of sample code F8 and 5 µg/mL of positive control, Cipladine and incubated for 24 h. Cipladine is a standard drug that is used in wound healing. Untreated cells were negative control. The cell migration and morphological changes of cells were observed in the images taken by inverted microscope, equipped with digital camera. The experiments were performed in triplicate (n % 3). The width of the scratch and wound closure at different time intervals (0, 48hrs) was analyzed by SAGLO software.

Groups	0 Hrs (mm)	48 Hrs (mm)
Control	00	47
Standard Cipladine (2 miro gram/ml)	00	67
Samples: Cream	00	50

Table: Percentage (%) of cells migrated towards the wound and involved in wound Closure

Microscopical images representing the In Vitro wound healing nature of sample. Cream: L929 cells were incubated in presence or absence of samples: Cream and Standard drug Ciplandine and images were captured at o and 48 Hrs.


Standard (Cipladine)


Sample:Gel:(1000 µg/mL)

All the concentration (1000micro gram/ml). Samples:Gel: Showed the good wound healing activity as compared to standard.

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