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FORMULATION & EVALUATION OF ANTIMICROBIAL HERBAL CREAM CONTAINING EXTRACT OF LEAVES/PLANT GENIUS VERNONIA

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Abstract: In the face of escalating antimicrobial resistance, there is a pressing need for novel therapeutic approaches. Traditional herbal medicine presents a promising avenue for exploration due to its historical use and reported pharmacological properties. This study investigates the antimicrobial potential of a semi-solid formulation comprising ethanolic extracts from Vernonia cinerea, alongside other medicinal plants commonly used in Ayurvedic medicine, against a panel of pathogenic fungi and bacteria. Vernonia cinerea, also known as Sahadevi, has been traditionally utilized in treating various ailments, exhibiting diverse pharmacological activities including antibacterial effects. Ten herbs, collectively known as "Dashapushpam," were selected based on their traditional use and screened for antimicrobial activity. The formulation was prepared by combining ethanolic extracts of these plants and oils in varying ratios. The antimicrobial efficacy of the combinations was evaluated through standardized assays. Results revealed promising antimicrobial activity against a spectrum of pathogenic fungi and bacteria, suggesting the potential of the herbal s emi-solid formulation as a natural alternative for combating microbial infections. This research underscores the importance of exploring traditional herbal remedies in the pursuit of novel antimicrobial agents, particularly amidst the current global challenge of antimicrobial resistance.

Index Terms-*Vernonia cinerea Linn, Antimicrobial activity, Soxhlet extraction, Petroleum ether extraction, Ethanolic extract, Hydroalcoholic solution, Phytochemical screening, Thin Layer Chromatography (TLC), Rf value, Quercetin, Herbal cream formulation, Agar well diffusion method, E. coli, S. aureus, Compatibility study (FT-IR), Flavonoids, Extract yield, Skin permeation test, Stability study, Homogeneity, Viscosity, Spread ability.*

I. INTRODUCTION

The pinnacle of antibiotic discovery in the 1960s, characterized by the identification of pivotal antibiotic classes such as tetracyclines, cephalosporins, aminoglycosides, and macrolides, contemporary challenges in microbial resistance threaten the efficacy of these therapeutic agents.[1][2][3] Vernonia cinerea, commonly referred to as Sahadevi, represents a constituent of dasapushpam, an herbal concoction comprising ten traditional medicinal plants prevalent in Kerala, India. Belonging to the Asteraceae family, Vernonia cinerea is a diminutive shrub found in Indian temperate regions and cultivated in China, recognized for its diverse pharmacological properties encompassing analgesic, anti-inflammatory, and antibacterial attributes, among others.[5][6][7][28] Ayurvedic medicine incorporates ten herbs collectively termed "Dashapushpam" where their antimicrobial potential against a spectrum of pathogenic fungi and bacteria has been scrutinized. Additionally, Baccharis trinervis and Vernonia cinerea are utilized in traditional medicine for combating various infectious diseases, prompting investigation into the antimicrobial

activity of their extracts. Chemical analyses indicate the presence of alkaloids, tannins, flavonoids, and various acids within Vernonia cinerea. Herbal medicine, denoting the utilization of plant-derived components for medicinal purposes, has garnered increased recognition owing to its efficacy in disease treatment and prevention.[8][9][43] With the World Health Organization reporting that a significant portion of the global population relies on herbal medicines for primary healthcare needs, attention has turned towards exploring herbal formulations for combating microbial infections.[2][7][41] Topical formulations, including antimicrobial creams and herbal skincare preparations, offer promising avenues for delivering therapeutic agents, leveraging the accessibility and non-invasive nature of skin administration.[10][13] Thus, this study endeavors to evaluate the antimicrobial efficacy of semi-solid herbal formulations derived from ethanolic extracts of local medicinal plants, including Vernonia cinerea, in various combinations, thereby addressing the contemporary challenge of microbial resistance.[2][9][12][45]

Fig.1 *Vernonia cinerea* Plant

Fig.2 Flowers of *Vernonia cinerea* plant

Table No 1. EXAMPLES OF ANTIMICROBIALS [14][15][42]

Clove	Antioxidant, antimicrobial, anti-inflammatory, anti-mutagenic, anti-allergic and anti-cancer.	Eugenol, eugenyl acetate, α-humulene, 2-heptanone, and β-caryophyllene
Portulaca	Antioxidant, antimicrobial, anti-inflammatory, anticancer, neuroprotective and antidiabetic.	Ascorbic acid, a-tocopherols, omega-3 fatty acids, apigenin, gallotannins, quercetin, and kaempferol
Tribulus	Antioxidant, antimicrobial, analgesic, anti-inflammatory and cardiovascular protective.	flavonoid, tannin and phenolic acids
Eryngium	Antioxidant, antimicrobial, anticancer, antidiabetic, antimalarial, anti-Alzheimer and anti-inflammatory.	Flavonoids, phenolic acids, and coumarins
Cinnamon	Antioxidant, antimicrobial, anti-inflammatory, anticancer, cholesterol-lowering, immunomodulatory and cardiovascular.	Cinnamaldehyde and eugenol
Turmeric	Antioxidant, antimicrobial, anti-inflammatory, anticancer, hypoglycemia and anticoagulant.	Vitamin-C, cineole, tumerone, borneol, zingiberene, d-sabinene, and d-phellandrene
Ginger	Antioxidant, antimicrobial, anti-diabetic, neuro- protective,	Phenolic acids, gingerols, paradols and

	analgesic, cardiovascular, gastrointestinal, anti-inflammatory, anticancer and antihypertensive.	shogaols
Thyme	Antioxidant, antimicrobial, expectorant, spasmolytic, mucolytic and antitussive.	Carvacrol, thymol and phenols
Pennyroyal	Antioxidant, antimicrobial, anti-hepatic, Anti-genotoxic.	Neo-menthol, pulegone and menthone
Fennel	Antioxidant, antimicrobial and anti-inflammatory	Phenolic compounds
Chamomile	Antioxidant, antimicrobial, anti-inflammatory, anticancer, analgesic, anti-hypoglycemic, anti-stress and hepatoprotective.	Flavonoids, terpenoids, phenolic compounds, apigenin and matricin
Mint	Antioxidant, antimicrobial, anticancer and anti-inflammatory.	Phenolic compounds
Burdock	Antioxidant, antimicrobial, anti-proliferative and anti-inflammatory.	Caffeic acid, rutin, o-hydrobenzoic acid, chlorogenic acid,
Eucalyptus	Antioxidant, antimicrobial anti-inflammatory and antipyretic.	Flavonols, hydroxybenzoic acids and hydrolyzable tannins
Primrose	Antioxidant, antimicrobial, anti-neuropathic, anti-inflammatory, anticancer and anti-ulcerogenic.	Phenolic acids, flavonoids, sterols, hydrocarbons, and tocopherols
Lemon balm	Antioxidant, antimicrobial, anti-stress, anti-Alzheimer, anti-inflammatory, anticancer, anti-cardiovascular and antispasmodic.	Phenolic componds such as thymol and carvacrol
Mallows	Antioxidant, antimicrobial, antinociceptive, anti-inflammatory, hepatoprotective and anticancer.	β-carotene, flavonoids, vitamin E, polyphenols, and vitamin C
Garlic	Antioxidant, antimicrobial, antidiabetic, anticancer, cardioprotective, anti-neurologica and anti-inflammatory.	Organosulfur such as Allicin, phenolic and polysaccharides compounds

II. Herbs Used in Cosmetics and Cosmeceuticals

The use of herbs in cosmetics and cosmeceuticals has been a practice for centuries, combining traditional knowledge with modern science to enhance beauty and skin health. This article explores several notable herbs, their chemical compositions, and their benefits for skin and hair care.[16][17][18][46]

Sr. No.	Name	Used in Cosmeceuticals	Skin Care Benefits
1.	Crocus Sativus (Saffron) **Vernacular Name:** Saffron **Family:** Iridaceae	Saffron, often referred to as Red Gold, is a perennial herb known for its luxurious and diverse chemical composition, including crocin, safranal, and picrocrocin. These compounds provide color, taste, and odor, respectively. Additionally, saffron contains volatile and non-volatile constituents such as lycopene, carotenes, terpenes, and their alcohols.	- Tan Removal: Helps in lightening skin tone. - UV Protection: Shields skin from harmful ultraviolet rays. - Antioxidant Properties: Protects skin from oxidative damage. - Treatment of Skin Ailments: Beneficial in managing various skin conditions.
2.	Camellia Sinensis (Green Tea) **Vernacular Name:** Green Tea **Family:** Theaceae	Green tea is derived from the leaves of Camellia sinensis, an evergreen plant predominantly grown in Southern Asia. The leaves are rich in flavonoids and catechins such as epicatechin, gallocatechin, epicatechin gallate, and proanthocyanidins.	- UV Protection: Prevents skin damage from UV exposure. - Anti-Tumor Properties: Reduces the risk of skin tumors. - Sebum Regulation: Inhibits 5-alpha-reductase, preventing dandruff and promoting hair growth in alopecia patients.
3.	Vitis Vinifera (Grape Seeds) **Family:** Vitaceae	Grape seeds from Vitis vinifera are rich in resveratrol, polyphenols, and flavonoids, with oils containing antioxidants like vitamins C and E, beta carotene, and various fatty acids. Grape seeds from Vitis vinifera are rich in	- Anti-Aging: Tightens skin and reduces signs of aging. - Acne Treatment: Provides astringent properties to manage acne. - Hyperpigmentation: Inhibits tyrosinase,

		resveratrol, polyphenols, and flavonoids, with oils containing antioxidants like vitamins C and E, beta carotene, and various fatty acids.	reducing melanin synthesis.
5.	Arctium Lappa (Burdock Root) **Vernacular Name:** Beggar's Buttons **Family:** Asteraceae	Burdock root is rich in polyacetylenes, fukinones, beta eudesmol, costusic acid, daucosterol, lappaol F, and neoarctin B. Its roots, fruits, and leaves have been historically used in Chinese medicine for dermatitis, acne, seborrhea, and as an antiseptic.	- Oil Control: Treats oily skin and prevents acne. - Detoxifying: Helps in detoxifying the skin. - Treats Psoriasis and Dandruff: Effective in managing these conditions.
6.	Tridax Procumbens (Coat-buttons) **Vernacular Name:** Tridax daisy **Family:** Asteraceae	Tridax procumbens, known for its traditional use in Ayurveda, contains fumaric acid, tannins, flavonoids, glucoluteolin, procumbenetin, and quercetin.	- Disease Treatment: Used for dysentery, bronchial problems, and diarrhea. - Hair Loss Prevention: Prevents hair loss and promotes hair growth.
7.	Allium Cepa (Onion) **Vernacular Name:** Onion **Family:** Amaryllidaceae	Onion bulbs contain albumin, allin, allyl propyl disulfide, allicin, allyl sulfides, zinc, magnesium, potassium, and calcium.	Hair Care Benefits - Baldness Treatment: Juice applied to the scalp promotes hair growth. - Mineral Benefits: Enhances oxygenation of red blood cells and oil secretion regulation.
8.	Nardostachys Jatamansi (Spikenard) **Vernacular Name:** Jatamansi **Family:** Valerianaceae	Jatamansi rhizomes are rich in jonon, 1,8 cineol, and bornyl acetate. This herb is known for promoting hair growth and has fungicidal and antibacterial properties.	Benefits - Hair Growth: Effective in treating alopecia induced by chemotherapy. - Skin Care: Treats fungal infections, dermatitis, psoriasis, and improves skin health.
9.	Terminalia Bellerica (Bibhitaki) **Vernacular Name:** Bibhitaki **Family:** Combretaceae	Bibhitaki, often used in combination with Emblica officinalis and Terminalia chebula, is known for treating various ailments like migraines, conjunctivitis, and alopecia.	- Constituents: Contains tannins, amino acids, glycosides, saponins, and flavonoids.
10.	Cuscuta Reflexa (Giant Dodder) **Vernacular Name:** Amar Bel **Family:** Cuscutaceae		Therapeutic Benefits Giant Dodder, a parasitic herb, contains coumarin, amarbelin, sitosterol, dulcitol, quercetin, and kaempferol. Hair Care Benefits - Alopecia Treatment: Inhibits 5-reductase enzyme, promoting hair growth.

III. Extraction Methods in Cosmeceutical Research

Extraction methods, also known as sample preparation methods, are crucial for isolating compounds of interest from natural sources. The choice of extraction technique significantly impacts the efficiency and quality of the extracted compounds. Extraction methods can be broadly categorized into traditional and modern techniques, each with unique advantages.[18]

A. Traditional Extraction Techniques:[19][20][44]

Sr. No.	Extraction Techniques	Extraction Process
1.	Liquid-Liquid Extraction (LLE)	Liquid-liquid extraction involves partitioning compounds between two immiscible liquid phases. It is widely used due to its simplicity and effectiveness in separating compounds based on their solubility in different solvents.
2.	Solid-Liquid Extraction (SLE)	Solid-liquid extraction, also known as maceration or percolation, involves extracting compounds from solid materials using a suitable solvent. This method is commonly used for its ease of operation and ability to handle large sample volumes.
3.	Solid-Phase Microextraction (SPME)	Solid-phase microextraction utilizes a solid adsorbent material to extract compounds from liquids or gases. It is valued for its solvent-free nature and ability to concentrate trace analytes.

B. Modern Extraction Techniques

Sr. No.	Extraction Techniques	Extraction Process
1.	Ultrasound-Assisted Extraction (UAE)	Ultrasound-assisted extraction employs ultrasonic waves to enhance the extraction process. The cavitation effect generated by ultrasound disrupts cell walls, facilitating the release of intracellular compounds. UAE offers reduced extraction times and higher yields compared to traditional methods.
2.	Pressurized Liquid Extraction (PLE)	Pressurized liquid extraction, also known as accelerated solvent extraction, utilizes high pressure and temperature to improve solvent penetration and solubilization of target compounds. PLE is efficient for extracting bioactive compounds from plant materials with reduced solvent consumption.
3.	Subcritical Water Extraction (SWE)	Subcritical water extraction uses water at temperatures between 100°C and 374°C and pressures sufficient to maintain the liquid state. This technique exploits water's altered properties under subcritical conditions to dissolve a wide range of compounds, offering an eco-friendly alternative to organic solvents.
4.	Supercritical Fluid Extraction (SFE)	Supercritical fluid extraction employs supercritical fluids, typically CO2, as solvents. Supercritical CO2 has tunable solvating properties, enabling selective extraction of compounds. SFE is noted for its efficiency, environmental friendliness, and ability to produce solvent-free extracts.
5.	Microwave-Assisted Extraction (MAE)	Microwave-assisted extraction uses microwave energy to heat solvents and samples, accelerating the extraction process. MAE offers rapid extraction times, high efficiency, and reduced solvent usage, making it suitable for thermolabile compounds.
6.	Instant Controlled Pressure Drop Extraction (DIC)	Instant controlled pressure drop extraction involves subjecting samples to a high-pressure environment followed by an instantaneous pressure drop. This method induces a rapid expansion and rupture of plant cells, enhancing the release of bioactive compounds. DIC is effective for extracting thermolabile and volatile compounds with high efficiency.

IV. Different Methods of Preparation of Creams

Creams are a fundamental component in cosmetics and personal care products, serving various functions from moisturizing to treating skin conditions. The preparation of creams involves careful selection and combination of ingredients, as well as specific

methods to ensure stability and effectiveness. This article discusses the preparation of oil-in-water (o/w) and water-in-oil (w/o) emulsion creams, along with the key ingredients used in their formulation.[20]

A. Preparation of Oil-in-Water (o/w) Emulsion Creams

Oil-in-water emulsions are characterized by a higher water content than oil. The preparation process involves the following steps:

1. **Melting the Oil Phase:** Oil-soluble components and emulsifiers are placed in a beaker and heated in a water bath to 75°C.
2. **Melting the Water Phase:** In a separate beaker, water, preservatives, and water-soluble components are heated to the same temperature.
3. **Combining Phases:** The heated oil phase is placed in a mortar and pestle, and the water phase is slowly added while triturating until a clicking sound is heard, indicating emulsification.
4. **Cooling and Adding Perfumes:** Once the mixture cools, perfuming agents and additional preservatives are added.[21]

B. Preparation of Water-in-Oil (w/o) Emulsion Creams

Water-in-oil emulsions have a higher oil content compared to water. The preparation involves similar steps but in reverse order for combining phases:

1. **Melting the Oil Phase:** Oil-soluble components and emulsifiers are heated in a beaker to 75°C.
2. **Melting the Water Phase:** Water and water-soluble components are also heated to 75°C in a separate beaker.
3. **Combining Phases:** The heated water phase is placed in a mortar and pestle, and the oil phase is slowly added while triturating until emulsification is complete.
4. **Cooling and Adding Perfumes:** Perfuming agents are added once the cream has cooled down.[21]

V. Key Ingredients in Cream Formulation:[13]

Sr. No.	Key Ingredients	Primary Functions
1.	Water	Water is the primary solvent used in cream formulations, dissolving other ingredients and forming emulsions. It must be free from toxins, pollutants, and microbes to ensure the safety and stability of the final product.
2.	Mineral Oils	Mineral oils, derived from petroleum, are clear, odorless, and highly refined. They are lightweight, inexpensive, and help reduce water loss from the skin, keeping it moisturized. Examples include light and heavy liquid paraffin and liquid petroleum
3.	Glyceride Oils	Glyceride oils, primarily vegetable oils, act as emollients and thickeners, forming a barrier on the skin surface to prevent water loss. Examples include almond oil, arachis oil, castor oil, coconut oil, and olive oil.
4.	Waxes	Waxes such as beeswax, carnauba wax, ceresin, and spermaceti are used to stabilize emulsions and increase the thickness of the lipid portion of creams. They help prevent the separation of oil and liquid components.
5.	Fats	Fats from animal, plant, or mineral origins are used to enhance the texture and emollient properties of creams. Common fats include lauric, margaric, palmitic, and stearic acids, as well as oleic acid for its unsaturated properties.
6.	Lanolin	Derived from wool fat, lanolin acts as a lubricant on the skin, providing a soft and smooth appearance. Hydrous lanolin contains 25%-30% water, while anhydrous lanolin has a higher melting point and a slight odor. Lanolin also helps form emulsions and blends well with other cosmetic ingredients.

7.	Colours	Originally derived from natural substances like turmeric and saffron, modern cosmetic colors are synthesized in laboratories for greater stability and consistency.
8.	Emollients	Emollients, or moisturizers, such as mineral oil, squalene, and lanolin, help soften and hydrate the skin by forming a protective barrier that retains moisture.
9.	Humectants	Humectants are hygroscopic compounds that attract and retain moisture, providing hydration and other benefits like exfoliation. Examples include glycerin, hydroxyethyl urea, betaine, sodium PCA, and sodium-L-lactate.
10.	Perfumes	Perfumes add a pleasant scent to creams, enhancing the user experience.
11.	Vitamins	Vitamins such as A, B, C, and E play crucial roles in maintaining skin health and are commonly included in cream formulations for their therapeutic benefits.
12.	Preservatives	Preservatives prevent microbial contamination and spoilage, ensuring the product's safety and longevity. Antioxidants may also be used to protect against oxidation. Synthetic preservatives are effective at low concentrations.

VI. Literature Review

1. Extraction of Secondary Metabolites

Avinash Kumar Jha et al. (2022) investigated the extraction of secondary metabolites, such as phenolic acids and flavonoids, highlighting the challenges posed by their insolubility. Conventional extraction techniques like Soxhlet, heat reflux, and maceration were identified as effective methods for isolating bioactive compounds, each utilizing distinct equipment. The study emphasized the importance of selecting an appropriate extraction method that optimizes product quality, process efficiency, cost-effectiveness, and environmental sustainability. Innovative extraction technologies, including high hydrostatic pressure (HHP), ultrasound (US), pulsed electric field (PEF), and supercritical fluid (SF), are gaining traction in the food industry. These methods are favored for their ability to increase extraction yields, reduce impurities, preserve thermo-sensitive compounds, and lower energy consumption compared to traditional methods.[30]

2. Topical Drug Development Guidance

Xuping Jin et al. (2022) discussed the guidance provided by the United States Food and Drug Administration (FDA) for the development of topical drugs. The guidance includes in vitro assessments of qualitative sameness (Q1), quantitative sameness (Q2), and physiochemical and structural characterization (Q3) of formulations. Critical quality attributes (CQAs) such as rheological properties, thermodynamic activity, particle size, globule size, and drug release/permeation rates are evaluated to ensure the desired product quality. The study explored the impact of various metamorphosis events on these CQAs, aiming to maintain consistent product performance.[36]

3. Synthesis and Characterization of Gold Nanoparticles

Lalita Singh et al. (2021) synthesized gold nanoparticles (AuNPs) using an aqueous extract from the aerial parts of Vernonia cinerea as a bioreducing agent. The color change from pale yellow to ruby-red indicated successful AuNP formation. Characterization was performed using UV–visible spectroscopy, X-ray crystallography (XRD), transmission electron microscopy (TEM), and energy dispersive X-ray analysis (EDX). The UV–Vis spectra showed a peak at 546 nm, confirming AuNP synthesis. The biosynthesized AuNPs demonstrated significant antimicrobial activity, particularly against Streptococcus pyogenes, outperforming the aqueous plant extract and showing better inhibition than ampicillin.[34]

4. Antioxidant and Antimicrobial Activities of Vernonia cinerea

Tushar Joshi et al. (2021) evaluated the DPPH radical-scavenging activity of Vernonia cinerea extracts, comparing them to standard antioxidants like gallic acid and quercetin. The extracts exhibited moderate antimicrobial activity against various pathogens, with zones of inhibition ranging from 9.0 to 13.5 mm. The minimum inhibitory concentration (MIC) and minimum

bactericidal concentration (MBC) for Candida albicans indicated high susceptibility, supporting the traditional use of V. cinerea in treating various ailments.[32]

5. **Traditional Medicinal Uses of Vernonia cinerea**

Liendo-Polanco et al. (2021) investigated the traditional medicinal uses of Baccharis trinervis and Vernonia cinerea, which are employed to treat diseases such as typhus, cholera, and malaria. Methanol-aqueous extracts of these plants were tested for antibacterial and antifungal activities using the disc-diffusion method. V. cinerea showed the broadest antimicrobial effect, validating its use in traditional medicine.[37]

6. **Formulation and Evaluation of Clove Extract Products**

Olubunmi J. Olayemi et al. (2021) formulated and evaluated creams and vaginal suppositories containing ethanol extract of clove. The extracts were prepared by macerating crushed clove buds in ethanol. The formulations were assessed for organoleptic properties, physicochemical characteristics, and antimicrobial efficacy. The products demonstrated significant antimicrobial activity against Gram-negative bacteria and Candida albicans, indicating the potential of clove extract in standardized dosage forms for treating vaginal infections.[47]

7. **Herbal Ingredients in Cosmetics**

Ritchu Babbar et al. (2020) emphasized the preference for herbal ingredients over chemical ones in cosmetics due to their availability and reduced side effects. Herbal ingredients are used in various formulations such as hair tonics, face creams, and skin care products. These natural components enhance beauty, improve skin texture, and treat conditions like dandruff and alopecia. The study highlighted the benefits of herbal ingredients in enhancing health and beauty through the maintenance of keratin structures and the generation of free radicals to boost collagen growth.[18]

8. **Pharmaceutical Creams for Wound Healing**

Chauhan Lalita et al. (2020) reviewed the applications of pharmaceutical creams for wound healing. These semi-solid preparations are used for cleansing, beautifying, moisturizing, and protecting the skin against infections. Medicated creams can accelerate the natural healing process and prevent infections in wounds. The review covered the wound healing process, methods of cream preparation, classifications based on function, and evaluation parameters for cream formulations.[13]

9. **Phytochemical Screening and Antimicrobial Activity**

Varsha V et al. (2016) conducted phytochemical screening and GC-MS analysis of Vernonia cinerea leaf extracts. The extracts were tested for antimicrobial activity against several bacterial strains. The ethanol and aqueous extracts showed significant activity, supporting the presence of bioactive compounds in V. cinerea leaves that contribute to its antimicrobial properties.[3]

10. **Antimicrobial Activities of Asteraceae Plants**

Daffodil ED et al. (2014) evaluated the antimicrobial activities of extracts from Emilia sonchifolia, Tridax procumbens, and Vernonia cinerea. The methanol extracts were tested against various bacterial, yeast, and fungal species. V. cinerea extract showed the most significant antimicrobial activity, suggesting its potential as a natural food preservative and treatment for human pathogens.[38]

11. **Anti-Bacterial Leaf Blight Activity**

Sasidharan S et al. (2009) tested different solvent extracts of Vernonia cinerea for their anti-Bacterial Leaf Blight (BLB) activity against Xoo bacterium. The study identified potential natural agrochemicals targeting the D-alanine-D-alanine ligase enzyme through in vitro and in silico approaches. The extracts demonstrated significant anti-BLB activity, indicating their potential use in sustainable agriculture.[39]

VII. Extraction Methods and Phytochemical Screening

A. Hot Continuous Extraction (Soxhlet) Method:[28]

Finely ground plant material placed in thimble within Soxhlet apparatus.

⇩

Solvent in flask is heated

⇩

Solvent vapors rise and condense

⇩

Condensed solvent drips into thimble

⇩

Compounds are extracted from plant material

⇩

Solvent collects and returns to flask

⇩

Process repeats until solvent is free of residue

⇩

Efficient extraction with less solvent

⇩

Significant time and cost savings, especially when scaled up

⇩

Sequential extraction with:

⇩

Petroleum ether and Ethanol

⇩

Hydroalcoholic solution

⇩

Determine percentage yield of each extract

i. **Defatting of Plant Material:** Dried and powdered plant material is extracted with petroleum ether at 50°C until the solvent is clear, indicating completion after 26 cycles.

ii. **Extraction with Ethanol:** The defatted material is extracted with 99.5% ethanol at 70°C for 26 cycles, producing a greenish-black gummy residue.

iii. **Extraction with Hydroalcoholic Solution:** The material is extracted with a 70:30 hydroalcoholic solution at 50°C for 10 cycles, yielding a brown-black extract.

iv. **Collection and Drying of Extracts:** Extracts are dried, ground, and sieved for further processing.

v. **Phytochemical and Preliminary Screening:**[22][23][24]

a. **Morphological Characterization:** The extract's odor, color, and taste are examined, and the percentage yield is calculated.

b. **Phytochemical Screening:**

- **Solubility:** Determined by dissolving 5-10 mg of the ethanolic extract in solvents like chloroform, water, petroleum ether, methanol, ethanol, and acetone.

- **Preliminary Tests for Chemical Constituents:**[21][29][33][40]

- **Flavonoids:** Detected using the Shinoda test, lead acetate test, alkali test, and heat test with zinc and HCl.

- **Carbohydrates:** Identified by Molisch's test, observing a red to violet ring formation.

- **Glycosides:** Detected by adding aqueous sodium hydroxide or using Million's test, resulting in yellow or brick-red precipitates.

- **Tannins:** Identified using the potassium dichromate test, observing a red precipitate.
- **Steroids:** Detected by Salkowski reaction, indicated by a red chloroform layer and greenish-yellow fluorescence.
- **Saponins:** Identified by the foam test, where persistent foam indicates their presence.

B. Thin Layer Chromatography (TLC) Profile:[27]

i. Sample Preparation for TLC

A few milliliters of the extract were dissolved in 10 ml of methanol and sonicated as needed for spotting on the TLC plate.

ii. Preparation of TLC Plate

Silica GF254 used as the absorbent

⇩

Measure approximately 25 g of silica gel GF254

⇩

Mix with 35 ml of distilled water in a glass mortar

⇩

Stir until homogenous

⇩

Add an additional 15 ml of distilled water

⇩

Stir to mix thoroughly

⇩

Transfer resulting suspension to a 150 ml glass flask

⇩

Shake vigorously for 2 minutes

⇩

Immediately spread suspension on the TLC plate using the pouring technique

⇩

TLC plate prepared

iii. Drying and Storage of Plates

The freshly coated TLC plates were air-dried until the transparency of the silica layer disappeared. The plates were then placed in a hot air oven and activated at 110-120°C for 30 minutes. This step ensures the removal of water and the activation of absorbent sites for better resolution and separation during chromatography.

iv. Application of Sample

Samples were applied to the TLC plates using capillaries, forming distinct spots on the plates.

v. Chromatographic Chamber Setup

A rectangular glass chromatographic chamber (16.5 cm × 29.5 cm) was used. To ensure proper chamber saturation and avoid edge effects, a smooth sheet of filter paper (approximately 15 × 40 cm) was placed in the developing chamber in a U-shape and soaked in the developing solvent. Once moistened, the paper was pressed against the chamber walls to adhere properly. The experiment was conducted at room temperature under diffused daylight.[27][29][38]

vi. Characterization and Identification of the Fractions

Fractions obtained from Vernonia cinerea extract were analyzed based on their chromatographic patterns. Given the complex mixture of various phytochemicals present in the Vernonia cinerea extract, complete separation was challenging. To address this, the plant extract was fractionated using different organic solvents.[35][40]

The chromatographic patterns of each fraction were meticulously examined, and fractions displaying similar TLC patterns were pooled together. These isolated fractions were then subjected to chemical tests to elucidate their chemical structures.[25][26]

VIII. List of Excipients

Sr. No.	Excipients	Function
1.	Stearic acid	Base
2.	Triethanolamine	Emulsifying agent
3.	Glycerin	Spreadability agent
4.	Borax	Base
5.	Bees Wax	Base
6.	Liquid Paraffine	Base
7.	Methyl Paraben	Preservative
8.	Propyl Paraben	Preservative

IX. Material and Equipment:

Sr. No.	CHEMICAL MATE4RIAL	SOURCE
1.	Bees wax	Oxford Lab Fine Chem LLP
2.	Liquid Paraffin	Samar Chemicals
3.	Stearic acid	Samar Chemicals
4.	Borax	Samar Chemicals
5.	Triethanolamine	Samar Chemicals
6.	Methyl Paraben	Samar Chemicals
7.	Propyl Paraben	Samar Chemicals
8.	Agar Agar Powder	Samar Chemicals
9.	Petroleum Ether	Oxford Lab fine Chem
10.	Ethanol	Oxford Lab Fine Chem
11.	Hydrochloric acid	Oxford Lab Fine Chem
12.	Anhydrous disodium phosphate	Oxford Lab Fine Chem
13.	Potassium dihydrogen phosphate	Oxford Lab Fine Chem
14.	Lavender Oil	Earth n Pure Store

X. Equipment:

Sr. No.	NAME OF THE INSTRUMENTS	COMPANY NAME
1.	Soxhlet Apparatus	Garg Process Glass Ind. Pvt. Ltd.
2.	Heating Mantle	Samar Chemiacals
3.	FT-IR Spectrophotometer	FTIR-8400S, SHIMADZU
4.	UV Visible Spectrophotometer	UV-1800, SHIMADZU Spectrophotometer
5.	Muffle Furnace	HICON Pvt. Ltd.,

6.	Hot Air Oven	HICON Pvt. Ltd.,
7.	Autoclave	HICON autoclave
8.	Laminar Air Flow	Grover Enterprises
9.	Incubator	HICON B.O.D. Incubator
10.	Magnetic Stirrer	HICON Magnetic stirrer
11.	Mechanical stirrer	Rajendra Electricals. Ind. Ltd.
12.	Franz Cell Diffusion Apparatus	Samar Chemicals
13.	Digital pH Meter	Systronics Model No.111E
14.	Biological Membrane	Merck Life Science Pvt. Ltd.
15.	Digital Weighing balance	Wensar, Model No. AW-220 and BX-620S
16.	UV Fluorescence Analysis cabinet	HICON Pvt. Ltd.

XI. Result and Discussion

A. Percentage Practical Yield:

Sr. No.	Extracts	Percentage of Practical Yield
1.	Petroleum Ether Extract	8.21 % W/W
2.	Alcoholic Extract	10.07 % W/W
3.	Hydroalcoholic Extract	6.43 % W/W

B. Phytochemical Analysis of Vernonia Cinerea Leaves Extract

Sr. No.	Phytoconstituents	Chemical tests performed	Result		
			Pet. Ether extract	Ethanolic extract	Hydroalcoholic extract
1.	Carbohydrates	a. Molish's Test	+	+	+
2.	Alkaloids	a. Dragendorff's Test	-	+	+
		b. Mayer's Test	-	+	-
3.	Steroids	a. Salkowoski Test	-	+	+
4.	Proteins	a. Biuret test	-	+	+
		b. Ninhydrin test	-	+	+
		c. Millon's test	-	+	-
5.	Saponins	Foam test	-	-	+

6.	Volatile oils	Sudan red test	-	-	-
7.	Glycosides	a. Borntrager test	-	-	-
		b. Modified Borntrager Test	+	+	+
		c. Killer Killani test	+	+	+
		d. Legal test	+	+	+
8.	Tannins andPhenolic compounds	a. Ferric Chloride test b. Lead acetate Test c. Gelatin test	-	+ + -	+ + -
9.	Flavonoids	Shinoda test	-	+	+

(+) test is Positive; (-) test is Negative

C. TLC Study:

Sr.No	Phytoconstituents	Solvent system	Detecting agent	SpotRf value	Color
1.	Alkaloids	Chloroform: Ethanol (95: 5)	UV Hager's reagent	0.31 0.31	Green Brown
2.	Glycosides	Ethyl acetate: ethanol: Water (81:11:8)	UV	0.51	Green
3.	Tannins	Alcohol: Chloroform (1: 9)	UV Vanillin Sulphuric Acid	0.72 0.58 0.31	Light Green Blue
4.	Steroids	Chloroform: Acetic acid: Ethanol (65:30:05)	UV Ammonia	0.58 0.61	Red Blue
5.	Proteins	Butanol: Acetic Acid: Water (4:1:5)	Ninhydrin reagent	0.47	Red
6.	Flavonoids	Ethyl Acetate: FormicAcid: glacial acetic acid: water (100:11:11:26)	UV	0.46	Green
7.	Resins	Petroleum Ether: Diethylether (75:25)	UV	0.31	Brown
8.	Terpenes	Butanol: Acetic Acid: water (4:1:5)	Bromine water	0.94	Green

D. Rf Value of Isolated Compound and Standards:

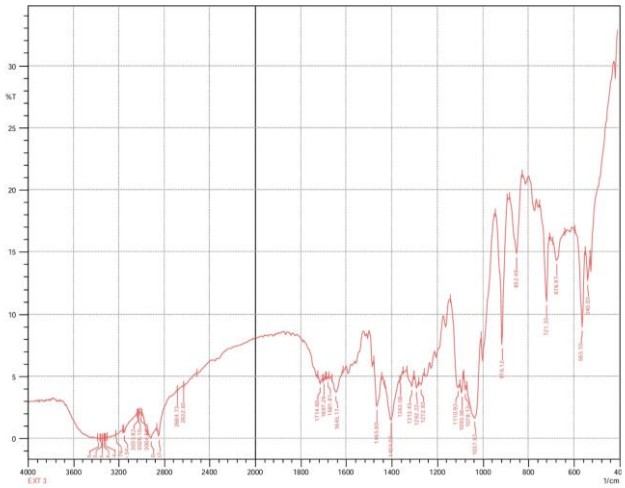
Sr. No.	Sample	Mobile phase	No. of spots	Rf value
1.	Isolated Compound	Toulene: Ethyl Acetate; Formic Acid	2	0.75, 0.64
3.	Standard Quercetin	Toulene: Ethyl Acetate; Formic Acid	1	0.76

E. Physicochemical screening:

Sr. No.	Parameters	Observation
1.	Loss on drying	13.20 % W/W
2.	Total ash value	13.78 %
3.	Acid- insoluble ash value	01.32 gm
4.	Water- soluble ash value	06.20 gm
5.	Sulphated ash value	06.59 gm
6.	Extractive value in petroleum ether	01.38 % W/W
7.	Extractive value in alcohol	18.68 %W/W
8.	Extractive value in hydroalcoholic solvent	14.96 % W/W

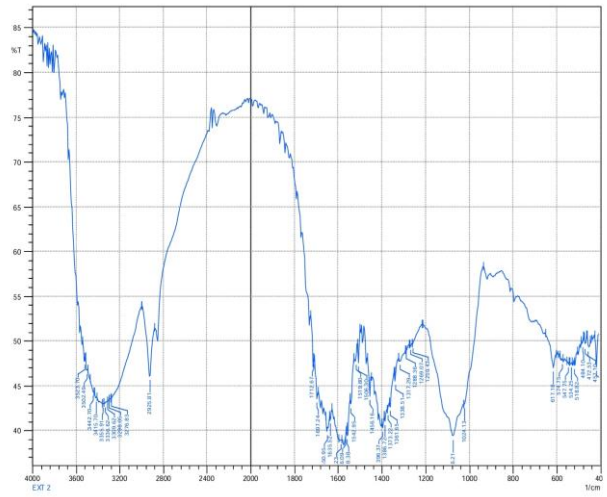
F. FT-IR studies of Pet. Ether Extract

Sr. No.	Reported value (cm^{-1})	Observed value (cm^{-1}) of Petroleum Ether Extract	Type of Vibration
1.	1300-800 cm^{-1}	916.12 cm^{-1}	C-O Str
2.	1500- 1500 cm^{-1}	1363.58 cm^{-1}	N=O Bend
3.	1500-1450 cm^{-1}	1465.80 cm^{-1}	C-C=C asymmetric str
4.	1730-1650 cm^{-1}	1697 cm^{-1}	C=O Str
5.	3000-2800 cm^{-1}	2918.10 cm^{-1}	H-C-H Asymmetric & Symmetric Strech
6.	3100-3000 cm^{-1}	3033.82 cm^{-1}	C=C-H asymmetric str
7.	3600-3100 cm^{-1}	3344 cm^{-1}	O-H Str



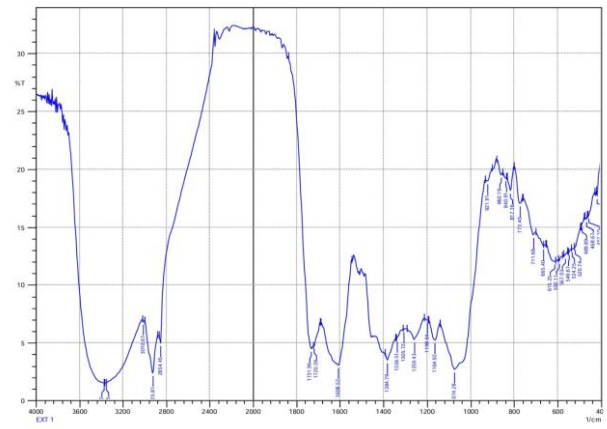
Graph of FT-IR Study of Petroleum Ether Extract

Sr. No.	Reported Value (cm^{-1})	served value (cm^{-1}) of Ethanolic Extract	Type of Vibration
1.	625-565 cm^{-1}	574.75 cm^{-1}	O=N Bend
2.	1300-1000 cm^{-1}	1269.07cm^{-1}	C-O Str
3.	1400-1300 cm^{-1}	1373.22 cm^{-1}	O=N Bend
4.	1500-1440 cm^{-1}	1446.16 cm^{-1}	H-C-H Bend
5.	1750-1625 cm^{-1}	1712.67 cm^{-1}	C=O str
6.	3400-2400 cm^{-1}	2925.81cm^{-1}	O-H Str
7.	3400-2400 cm^{-1}	3298.05 cm^{-1}	O-H Str
9.	3600-3100 cm^{-1}	3523.70 cm $^{-1}$	O-H Str



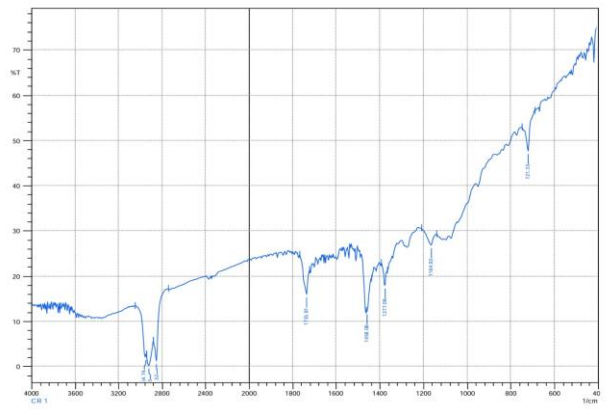
Graph of FT-IR study of Ethanolic extract

Sr. No.	Reported value (cm^{-1})	Absorption Ranges (cm^{-1}) of Hydroalcoholic Extract	Type of Vibration
1.	625-565 cm^{-1}	567.03 cm^{-1}	O=N Bend
2.	900-700 cm^{-1}	711.68 cm^{-1}	C-H Bend
3.	1400-1300 cm^{-1}	1384.79 cm^{-1}	O=N Bend
4.	1730-1650 cm^{-1}	1720.39 cm^{-1}	C=O Str
5.	1755-1650 cm^{-1}	1731.96 cm^{-1}	C=O str
6.	3600-3100 cm^{-1}	3352.05cm^{-1}	O-H Str



Graph of FT-IR study of Hydroalcoholic extract

Sr. No.	Reported value (cm^{-1})	Absorption Ranges (cm^{-1})	Type of Vibration
1.	625-565 cm^{-1}	574.75 cm^{-1}	O=N Bend
2.	1300-1000 cm^{-1}	1278.72cm^{-1}	C-O Str
3.	1755-1650 cm^{-1}	1716.53cm^{-1}	C=O str
4.	3000-2800 cm^{-1}	2918.10 cm^{-1}	H-C-H Sym and asym Str
5.	3400-2400 cm^{-1}	2954.74 cm^{-1}	O-H str
6.	3400-2400 cm^{-1}	3299.98 cm^{-1}	O-H Str
7.	3600-3100 cm^{-1}	3523.70 cm $^{-1}$	O-H Str



Graph of FT-IR study of Ethanolic Extract and O/W Cream Excipients

Sr. No.	Reported value (cm^{-1})	Absorption Ranges (cm^{-1})	Type of Vibration
1.	1300-1000 cm^{-1}	1164.92 cm^{-1}	C-O Bend
2.	1755-1650 cm^{-1}	1735.81 cm^{-1}	C=O Str
3.	3000-2800 cm^{-1}	2852.52cm^{-1}	H-C-H Sym and asymstr
4.	3400-2400 cm^{-1}	2921.96 cm^{-1}	O-H Str
5.	3400-2400 cm^{-1}	2954.74 cm^{-1}	O-H str

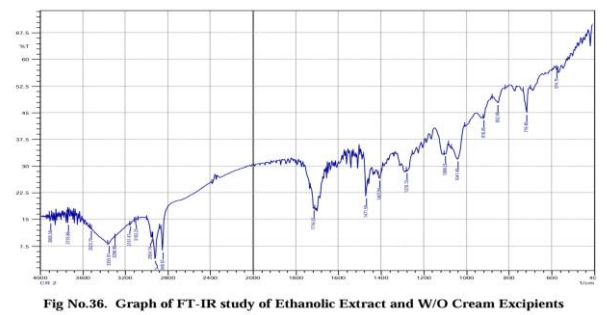


Fig No.36. Graph of FT-IR study of Ethanolic Extract and W/O Cream Excipients

Graph of FT-IR study of Ethanolic Extract and W/O Cream Excipients

G. Evaluation of Herbal Cream:

Sr. No.	Parameters	Observation	
		Herbal O/W Cream	Herbal W/O Cream
1.	Physical Appearance a) Colour b) Odour c) Texture	Faint greenPleasant Smooth	Green PleasantSmooth
2.	Spreadability	25.17 g.cm/s	28.45 g.cm/s
3.	pH determination	7.2	7.3
4.	Sap Value	27.1	25.3
5.	Acid Value	5.7	6.1
6..	Dye test	O/W	W/O
7.	Viscosity	19034 cps	23409 cps
8.	Homogeneity	Homogeneous	Homogeneous
9.	Removal	Easily remove after wash	Easily remove afterwash
10.	After feel	Soft	Emolient
11.	Type of Smear	Thin film	Thin film

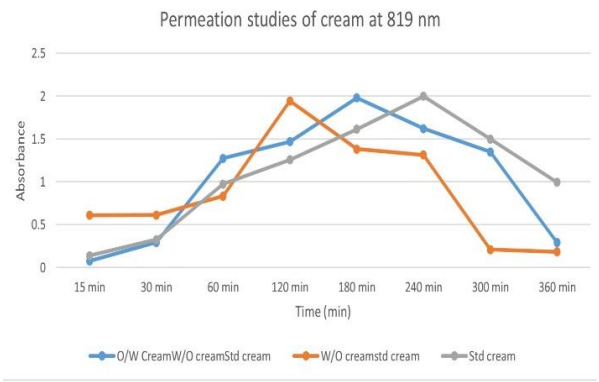
H. Stability Studies of Cream:

Sr. No.	Tests	O/W Herbal cream		W/O Herbal cream	
		Before 30 days	After 30 days	Before 30 days	After 30 days
1.	Physical Appearance	Semisolid	Semisolid	Semisolid	Semisolid
2.	Texure	Smooth	Smooth	smooth	Smooth
3.	Colour	Faint green	Faint green	Faint green	Faint green
4.	Odour	Pleasant	Pleasant	Pleasant	Pleasant
5.	pH value	7.2	7.1	7.3	7.4
6.	Viscosity	19034 cps	19189 cps	23409 cps	23278 cps
7.	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
8.	Spreadability	25.17 g.cm/s	26.67 g.cm/s	28.45 g.cm/s	30.79 g.cm/s
9.	Removal	Easily remove	Easily remove	Easily remove	Easily remove

I. Study of Skin Permeation Test of Formulations:

Skin Permeation Test

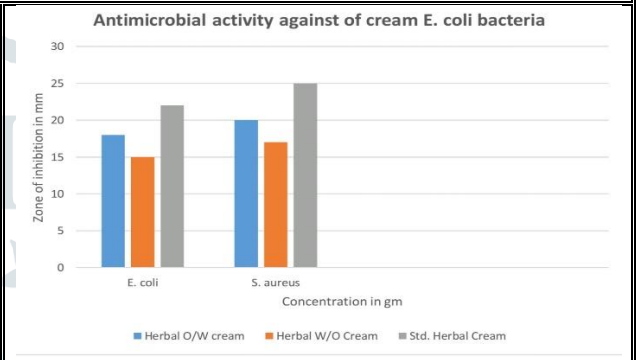
Sr. No.	Time (min)	Absorbance of Std.Cream (819 nm)	Absorbance of O/WCream (819 nm)	Absorbance of W/O Cream (819 nm)
1.	15 min	0.138	0.075	0.608
2.	30 min	0.326	0.293	0.612
3.	60 min	0.973	1.272	0.832
4.	120 min	1.256	1.467	1.943
5.	180 min	1.612	1.978	1.380
6.	240 min	1.997	1.620	1.312
7.	300 min	1.498	1.349	0.208
8.	360 min	0.994	0.290	0.181



Graph of Permeation study of cream at 819 nm

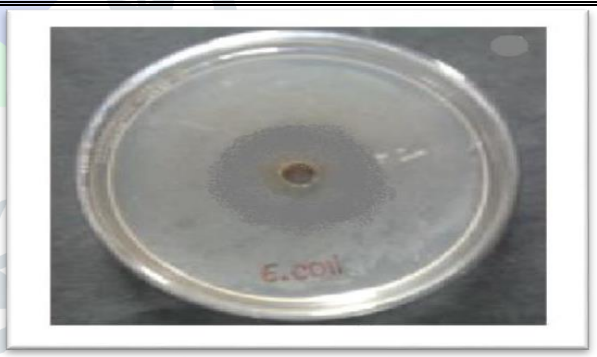
Antimicrobial activity of Herbal cream and Std. Cream

Sr. No.	Type of Cream	Concentration (gm)	Zone of inhibition of Cream (mm)	
			E. coli	*S. aureus*
1.	Herbal O/W Cream	1 gm	18 mm	20 mm
2.	Herbal W/OCream	1 gm	15 mm	17 mm
3.	Std. HerbalCream	1 gm	22 mm	25 mm

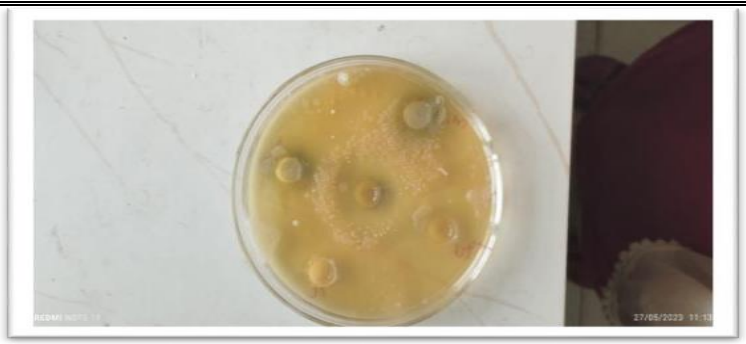


Antimicrobial activity of cream

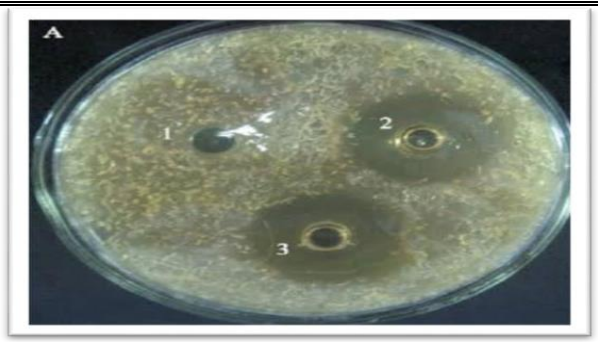
Zone of Inhibitionagainst E. coli bacteria
A. Herbal O/W Cream
B. Herbal W/O Cream



Zone of Inhibition ofStandard herbal cream against E. coli bacteria



Zone of Inhibition against
S. aureus bacteria Std. herbal cream



Zone of Inhibitionagainst *S. aureus* bacteria
A. Herbal O/W Cream
B. Herbal W/O Cream

XII. Summery and Conclusion:

The leaves of Vernonia cinerea Linn were gathered, dried, and authenticated. The dried leaves were then ground into coarse material for use in experiments. A total of one hundred grams of the plant material was accurately measured. Following the work plan, the leaves of Vernonia cinerea Linn underwent successive extraction, starting with defatting using petroleum ether. The defatted residue was then refluxed with 99.5% ethanol for 12 hours. The resulting ethanolic extract was further processed with a 30:70 hydroalcoholic solution. The plant was extracted using various organic solvents in increasing order of polarity, and the percentage yield was calculated.

The extracts were evaluated for antimicrobial activity using the Agar well diffusion method, with the alcoholic extract showing the highest activity against E. coli and S. aureus. This led to the formulation of an antimicrobial herbal cream using the alcoholic extract, which was then assessed for its antimicrobial properties. Preliminary phytochemical screening indicated positive results in Molisch, Shinoda, Alkali, Lead acetate, and Salkowski tests.

Thin Layer Chromatography (TLC) studies were conducted on the extracts, with Rf values calculated using various mobile phases, including Chloroform: ethanol (95:5), Ethyl acetate: ethanol: water (81:11:8), Chloroform: acetic acid: ethanol (65:30:05), and Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26). The preliminary phytochemical analysis of different solvent extracts was performed, and Rf values were compared with the standard flavonoid, quercetin.

Herbal O/W and W/O creams formulated using the ethanolic extract of V. cinerea showed superior antimicrobial activity compared to other extracts and were evaluated against E. coli and S. aureus. A compatibility study (FT-IR) of herbal extracts and cream formulation indicated no significant changes in absorbance, confirming compatibility. The antimicrobial activity of the herbal cream demonstrated a better zone of inhibition against both gram-negative and gram-positive bacteria. The formulated creams were assessed for physical appearance, pH, homogeneity, viscosity, spreadability, skin permeation, and stability.

In conclusion, the leaves of Vernonia cinerea Linn exhibited significant antimicrobial activity in the 99% alcoholic extract, with an alcohol-soluble extractive value of 3.6% w/w. The flavonoid-rich fraction was compared with quercetin, showing comparable results. The highest percentage yield of extracted plant material was found with ethanol. The formulated herbal F3 batch of O/W cream and F4 batch of W/O cream using the alcoholic extract demonstrated enhanced antimicrobial activity. Evaluation parameters such as physical appearance, pH, homogeneity, viscosity, and spreadability showed satisfactory results. The skin permeation test indicated better absorbance through the semipermeable membrane. Finally, a 30-day stability study showed no significant variations in visual appearance, nature, fragrance, or phase separation.

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