World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 8.21 Coden USA: WJPLA7

A REVIEW OF FORMULATION AND EVALUATION OF ANTIOXIDENT CREAM USING DIFFERENT PLANT EXTRACT

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DOI: https://doi.org/10.5281/zenodo.20526759

How to cite this Article: D. Preethi*. (2026). A Review of Formulation and Evaluation of Antioxident Cream Using Different Plant Extract. World Journal of Pharmaceutical and Life Sciences, 12(6), 239–250.

Article Received on 05/05/2026 **Article Revised on 25/05/2026** **Article Published on 03/06/2026**

1. INTRODUCTION

Antioxidants are chemicals that stop or slow oxidation by neutralizing free radicals in the body. Oxidation is a natural process that happens during metabolism, resulting in unstable chemicals known as free radicals. These free radicals can harm cells, proteins, lipids, and DNA, contributing to a variety of diseases and aging. Antioxidants keep the body healthy by donating electrons to free radicals and lowering oxidative stress.

Antioxidants, which are naturally found in fruits, vegetables, herbs, medicinal plants, and cereals, have recently grown in popularity in the fields of pharmacy, medicine, food science, and cosmetics due to their ability to protect against chronic diseases. Chemically synthesized antioxidants are also available for industrial use. Antioxidants occur naturally in fruits, vegetables, herbs, medicinal plants, and cereals. Chemical processes are also used to create synthetic antioxidants for industrial usage.

Oxidative stress is induced by an imbalance between free radical production and antioxidant defences. Cancer, diabetes, cardiovascular disease, neurological disorders, arthritis, and liver disease have all been linked to elevated oxidative stress. Antioxidants help to maintain good health, boost immunity, and reduce cellular damage. As a result, antioxidant research has become a major focus in pharmaceutical and biological sciences.

Free Radicals

Free radicals are highly reactive molecules containing one or more unpaired electrons. Because of their unstable nature, they react rapidly with nearby molecules.

Sources of Free Radicals

- Normal metabolism
- Smoking
- Pollution
- Radiation
- Stress
- Alcohol consumption
- Certain drugs
- Ultraviolet rays

Types of Free Radicals

Reactive Oxygen Species (ROS)

- Superoxide radical
- Hydroxyl radical
- Hydrogen peroxide

Reactive Nitrogen Species (RNS)

- Nitric oxide
- Peroxynitrite

Free radicals can damage biological molecules and contribute to oxidative stress.

Oxidative Stress

Oxidative stress is a condition in which free radical production exceeds the body's antioxidant defence system.

Effects of Oxidative Stress

- Damage to cell membranes
- DNA mutation
- Protein denaturation
- Lipid peroxidation
- Tissue injury

Long-term oxidative stress is associated with aging and

many chronic diseases.

Antioxidants
Antioxidants are compounds that inhibit oxidation and protect cells from free radical damage.

Mechanism of Action
Antioxidants act by
1. Scavenging free radicals
2. Donating electrons or hydrogen atoms
3. Chelating metal ions
4. Breaking oxidation chain reactions
5. Enhancing antioxidant enzymes

Characteristics of Antioxidants
- Reduce oxidative stress
- Protect tissues
- Prevent cell damage
- Improve immunity
- Delay aging

Classification of Antioxidants
Based on Source Natural Antioxidants
Obtained from natural sources such as fruits, vegetables, herbs, and medicinal plants. Examples:
- Vitamin C
- Vitamin E
- Flavonoids
- Polyphenols
- Carotenoids

Synthetic Antioxidants
Prepared chemically and mainly used in food and pharmaceutical industries. Examples:
- BHT
- BHA
- Propyl gallate

Based on Solubility
Water-Soluble Antioxidants
- Vitamin C
- Glutathione

Lipid-Soluble Antioxidants
- Vitamin E
- Beta-carotene

Natural Antioxidants
Natural antioxidants are safer and widely preferred because of their therapeutic benefits.

Plant-Derived Antioxidants
Medicinal plants contain antioxidant phytochemicals such as
- Flavonoids
- Phenolic compounds
- Tannins
- Alkaloids
- Terpenoids

Sources of Natural Antioxidants Fruits
- Orange
- Grapes
- Pomegranate
- Strawberry

Vegetables
- Tomato
- Spinach
- Carrot

Herbs and Spices
- Turmeric
- Ginger
- Tulsi
- Green tea
- Garlic

Natural antioxidants help in preventing oxidative damage and improving health.

Synthetic Antioxidants
Synthetic antioxidants are chemically prepared substances used to prevent oxidation in food and pharmaceutical products.

Advantages
- Stable
- Economical
- Easily available

Disadvantages
- Possible toxic effects
- Safety concerns on prolonged use

Because of these limitations, natural antioxidants are increasingly preferred.

Advantages of Natural Antioxidants
- Safe and effective
- Less toxic
- Easily available
- Eco-friendly
- Multiple therapeutic benefits

Natural antioxidants are increasingly used in herbal formulations.

Limitations of Antioxidants
- Poor stability
- Low bioavailability
- Variation in plant composition
- Difficulty in standardization

Despite limitations, antioxidants remain important therapeutic agents.

Free radicals have been known to cause skin cancer, photodamage, and accelerated aging resulting from excessive exposure to sunlight and oxidative stress. The human body produces antioxidants in limited quantities, which may not suffice to counteract the daily production of free radicals. Hence, external antioxidant intake is

required to combat free radicals.[1] Antioxidants neutralize free radicals and stop the oxidation of biological molecules such as lipids, proteins, and nucleic acids, protecting the skin from further damage and slowing aging. Furthermore, dietary adjustments and topical preparations with various active components, such as antioxidants, counteract these harmful effects.[2,3]

Topical antioxidants improve the skin's biophysical parameters, making the skin healthy and free from infections and conditions associated with oxidative stress.[4,5] Developing effective and stable topical formulations faces several challenges, including efficient skin and epidermal penetration and maintaining antioxidant potency by minimizing oxidative damage due to ionization, pH extremes, high storage temperatures, and metal ions.[6,7]

Typically, topical formulations like creams and gels are chosen to address skin issues such as premature aging. These formulations, including creams, gels, and face masks, are commonly used by the public. Generally, these dosage forms' formulation and physical quality should conform to standards and exhibit good physical quality. However, these topical dosage forms still have their limitations. Gels struggle with delivering hydrophobic drugs, which are easily lost when exposed to sweat or water.[8] Creams require a heating process during manufacturing and can break down if the formula is incorrect,[9] while ointment (hydrocarbon-based) shows greasiness as well as phase inversion.[10]

An innovative approach is needed to address the limitations of the previously mentioned topical dosage forms, such as utilizing an emulgel a gel-based emulsion.[8] Emulgel formation utilizes a combination of hydrogel and emulsion systems to create concrete structures that enhance the stability of the bioactive ingredient or delivery method. Other advantages of emulgel are incorporating hydrophobic drugs, better loading capacity, better stability, production feasibility, low dosage form cost, and improved patient compliance.[11,14] Common excipients used in emulgel include Carbopol 940 and stearic acid. Frequently utilized as a gelling agent, Carbopol quickly disperses in water at room temperature, exhibits a wide viscosity range, and achieves the desired gel base viscosity with only a small concentration.[15,16] On the other hand, stearic acid is a versatile excipient commonly used in topical formulations and widely used in cosmetics. Stearic acid is frequently used in cream formulations when partially neutralized with alkalis or triethanolamine. This results in the generation of a creamy base when combined with 5-15 times its weight in aqueous liquid. The appearance and plasticity of the cream depend on the proportion of alkali utilized.[16] To maintain the stability of emulgel preparations, it is crucial to use the appropriate excipients at the correct concentration. It includes emulsifiers, bases, and gelling agents.[17] Carbopol and stearic acid function as gelling agents and bases, which determine the stability of the emulgel.

As technology progresses, various plants have demonstrated anti-inflammatory, antioxidant, anticancer, and other beneficial effects in numerous studies. Asiaticoside in Centella asiatica L. (also known as Gotu kola) and β-sitosterol in Moringa oleifera have antioxidant activity, a potential active ingredient in anti-aging dosage forms.[18–22] Gotu kola contains vitamins C and B, with its main active ingredient being triterpenoid glycosides like asiatic acid, asiaticoside, madecassic acid, madecassoside, sitosterol, kaempferol, and polyacetylene compounds.[19] This plant is recognized for its antioxidant and ethanol extract antioxidant activity against the DPPH free radical of 79.49 (%), corresponding to 6.059±0.022 mg ascorbic acid equivalent/g DW has been reported.[23] Moreover, Moringa plants, particularly their leaves, contain high antioxidants, including crucial phenolic bioactive compounds like flavonoids (quercetin, kaempferol, isorhamnetin, and apigenin).[24] Research has indicated that Moringa leaves contain β sitosterol at 90 mg/g, total phenolics at 8 µg/mL, flavonoids at 27 µg/mL, and high antioxidant activity (69.72±1.15%) of the ethanol extract.[25,26] The Gotu kola herb extract and Moringa leaf extract have antioxidant activity. Combining the two extracts has the potential to increase their antioxidant activity. Studies have shown that combining extracts increases antioxidant activity compared to each extract alone.[30] A study by Reubun et al. showed that combining Moringa oleifera and Centella asiatica extracts can help cure Alzheimer's disease more effectively.[31] This study will combine Gotu kola and Moringa leaf extracts to formulate an emulgel with improved antioxidant activity. Previous studies have shown promising results for this combination of active ingredients. Sohail et al.3 reported that the emulgel formulation based on lycopene significantly improved skin hydration and elasticity over 12 weeks. Additionally, compared to the placebo formulation, there were significant reductions in erythema, melanin, and sebum content. So far, researchers have not found any reports regarding the topical effects of lycopene-based emulgel on human volunteers. This supports the importance of formulating emulgel dosage forms with anti-aging potential. Previous studies on emulgel formulations as antioxidants have demonstrated their potential to promote topical anti-aging, limit oxidative stress-related skin disorders, and prevent premature aging process.[32–34]

In this study, an emulgel dosage form was formulated by combining extracts from Gotu kola and Moringa leaves, intended for anti-aging purposes, and subjected to physical quality testing. Numerous studies have been conducted on the antioxidant activity of Gotu kola extract and Moringa leaf extract, as evidenced by published scientific articles. One study has even formulated Moringa leaf extract into an emulgel.[34] No research has published results on emulgel formulations

using active ingredients from a combination of Gotu Kola and Moringa leaf extracts, specifically for anti-aging purposes. This research aimed to determine the appropriate concentration of Carbopol and stearic acid in the emulgel formula to produce a dosage form with the best physical quality and antioxidant activity. The objective was to assess the emulgel's physical quality in line with established standards, thereby addressing the limitations of other topical dosage forms. It represents a novel advancement in pharmaceutical dosage forms aimed at combating aging. In addition to using a combination of Gotu kola herb extract and Moringa leaf extract as the active ingredients, the novelty of this study lies in the process of making this emulgel, which differs from that of previous researchers, specifically in the development and mixing of the gelling agent with the emulsion base.

Key traditional therapeutic herbal strategy exploits the combination of several medicinal herbs to achieve extra therapeutic effectiveness. This is known as polyherbalism. Polyherbal formulations are mixture of herbs, prepared in a number of formulations such as decoctions, elixirs, infusions, creams, gels, ointments and paste etc, in order to achieve Maximum therapeutic efficacy.[35] Polyherbal formulations contain plant-based pharmacological agents which may exert synergistic, potentiate, agonistic or antagonistic actions by virtue of its diverse active principles within themselves.[36,37] These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects.[35] The use of polyherbal formulations have been prominent in management of the effects of aging and improvement of skin tone. Aging is a natural progressive process that leads to aesthetic and functional changes in the skin[36] promoted by a group of molecules known as radicals. These radicals also known as reactive oxygen species can be created by combustion of by products and UV radiation interacting with the oxygen present in the skin.[37] In normal conditions a balanced equilibrium existing between these radicals and the skin's natural antioxidants such as vitamin E, co-enzyme 10Q, ascorbates, and carotenoids.[38] The excess generation of free radicals overwhelms the skins natural cellular antioxidants creating a condition which is known as oxidative stress. Oxidative stress leads to oxidative damage which manifest physically as aging, a process which can be effectively retarded by the use of externally applied antioxidants.[39]

Plant extracts possessing antioxidant properties have been explored in phytocosmetic field as they present molecules that could inactivate reactive oxygen species restoring skin homeostasis and preventing erythema and premature aging of the skin.[40] African walnut Tetracarpidium conophorum has a long history as food plant and is grown by farmers across West African rain forest. T. conophorum is widely distributed and consumed by the inhabitants of the Guinea Zone of West and Central Africa.[41] Studies have shown that the African walnut possesses some beneficial properties like antibacterial, antioxidant.[42,43] and immune-stimulating activities. It is commonly used in Nigerian folkloric medicine for the treatment of bacterial infections and ailments caused by oxidative stress.[42] Photochemical screening of ethanol extracts of T. conophorum showed presence of alkaloids, saponins, glycosides, flavonoids and tannins. Akomolafe et al.[44] evaluated the anti-peroxidative activity of the leaves of T. conophorum by determining their capacity to reduce malondialdehyde levels. The results suggest that the extract from T. conophorum leaves had greater capacity to reduce lipid peroxidation and thus, this plant may be useful in the treatment/management of cellular damage involving reactive oxygen species. Amaeze et al.[45] evaluated the antioxidant activity of T. conophorum extracts of fresh and dried leaves. The result revealed that ethanol extract of the dried leaves had high antioxidant as well nitric oxide radical inhibition activity comparable to that of rutin and ferric reducing power.

Ocimum gratissimum is a herbaceous which belongs to the family Lamiaceae. It is commonly known as scent leaf. The plant is indigenous to India and West Africa. The essential oils contain eugenol, thymol and p-cymene which show some evidence of antioxidant, anti-bacterial, anthelminthic and insecticidal properties.[46] The antioxidant capacity of essential oils Ocimum spp. were evaluated using a HPLC-based hypoxanthine/xanthine oxidase and DPPH assays with strong antioxidant capacity being evident in all the oils.[47] Extracts from the leaves of O. gratissimum were investigated for their phytochemical constituents and antioxidant activity suggesting the rich phytochemical content of O. gratissimum and its good antioxidant activity.[47] The aim of this research work was to formulate and evaluate a polyherbal antioxidant face cream using the ethanol extracts of T. conophorum and O. gratissimum having known antioxidant activity that will protect the skin from the effects of reactive oxygen and free radicals as well as act as an emollient.

1. FOMULATION AND EVALUATION OF ETHANOLIC PLANT EXTRACT OF ANTIOXIDENT CREAM USING DIFFERENT PLANT EXTRACT

1.1 Formulation and Evaluation of Natural Antioxidant Cream Comprising Methanolic Peel Extract of Dimocarpus longan[48]

Photo aging is a common problem that occurs in our community due to ongoing exposure to ultraviolet rays. The use of antioxidants is an effective approach to prevent symptoms related to photo-induced aging of the skin. Thus, the present study was to prepare and evaluate the antioxidant cream comprising the methanolic peel extracts of Dimocarpus longan for their radical scavenging activity. Antioxidant activity of peels and seeds methanolic extract (by continuous hot percolation-soxhletation) of D. longan was assessed by using stable 2,2 -Diphenyl-1-picryl hydrazyl (DPPH). The extract of

the D. longan fruits contained three major polyphenolic compounds which are corilagin, gallic acid, and ellagic acid which are responsible for the antioxidant properties. Methanolic extracts of both peels and seeds of D. longan exhibited high radical scavenging properties, the IC50 result revealed that peels extract was having higher antioxidant properties with 23.5 µg/ml compared to the seeds 32.13 µg/ml. Based on the higher scavenging activity the peels was chosen to prepare as formulation. Thus, the cream was formulated with 2.5% of peels extract by fusion method with incorporation of two different emulsifying agents for two formulations (F1 & F2). The evaluation of the formulations was done on different parameters like pH, spreadability, rheological study, non-volatile matter at 105 °C, physical stability of cream and microbial limit test. Both the formulations (F1 & F2) were showed good pH, homogeneity, appearance, ease to remove, good consistency, spreadability and no microbial growth. However, after four weeks of storage formulation of F1 showed cracking and phase separation. The evaluation parameters of the formulated cream F2 showed good results and are safe to use for skin. The present results indicates that the D. longan fruit peel extract has a good potential for cosmetic product development.

1.2 Polyherbal Antioxidant Topical Preparation Comprising Ethanol Extract of *Tetracarpidium conophorum* and *Ocimum gratissimum*[48]

The use of antioxidants is an effective approach to prevent symptoms related to photo- induced aging of the skin. The aim of this research work was to formulate and evaluate a polyherbal antioxidant face cream using the ethanol extracts of *Tetracarpidium conophorum* and *Ocimum gratissimum*. The ethanol extract of the herbs was incorporated at varying concentrations into six different emulsion bases. Antioxidant activity of the formulations was assessed using 2,2-diphenyl-1-picrylhydrazyl method. The formulations were evaluated for pH, viscosity, spreadability and microbial content. Accelerated stability tests were performed on all the formulations to assess stability at varying storage conditions. All the formulations showed good spreadability, good consistency, homogeneity, appearance, pH and no phase separation occurred. Non-Newtonian pseudo- plastic flow influenced by increased shear was experienced by all the formulations. Concentration dependent antioxidant activity was observed with FC2 and FC4 showing the highest antioxidant activity with IC_{50} value of 80.1 and 83.2 µg/ml, respectively. The polyherbal antioxidant preparation containing extracts of *T. conophorum* and *O. gratissimum* shown to exhibit excellent antioxidant properties. It can serve to protect the skin from reactive oxygen species created by UV radiation and environmental toxin, thus protecting the skin from photo aging.

1.3 Formulation and Evaluation of Polyherbal Antioxidant Face Cream Containing Ethanol Extracts of *Psidium Guajava* and *Ocimum Gratissimum*[49]

Background: Aging is a natural progressive process that leads to aesthetic and functional changes in the skin. The aim of this research work is to formulate and evaluate a polyherbal antioxidant face cream using the ethanol extracts of psidium guajava and ocimum gratissimum.

Method: The ethanol extract of the herbs was incorporated at varying concentrations into six different emulsion bases. Antioxidant activity of the formulations was assessed using 2, 2-Diphenyl-1-picrylhydrazyl method. The formulations were evaluated for pH, viscosity, spreadability and microbial content. Accelerated stability tests were performed on all the formulations to assess stability at varying storage conditions.

Results: All the formulations showed good spread ability, good consistency, homogeneity, appearance, pH without phase separation occurring. Rheological tests showed that all formulations exhibited non-Newtonian pseudo plastic flow. All six formulations also showed concentration dependent antioxidant activity. Ascorbic acid a potent antioxidant served as the standard for these tests. Formulation AFCR6 showed the highest antioxidant activity with IC50 value of 80.1 µg/mL.

Conclusion: The polyherbal antioxidant cream containing extracts of Psidium guajava and Ocimum gratissimum have been shown to have excellent antioxidants properties. It can serve to protect the skin from reactive oxygen species created by UV radiation and environmental toxin, thus protecting the skin from photo aging. **Keywords:** Antioxidant, Face cream, Psidium guajava, Ocimum gratissimum.

1.4 Formulation and evaluation of antioxidant cream based on markhamia tomentosa ethanolic extract and citrus sinensis oil[50]

Premature aging is a common problem that occurs globally due to continuous exposure to the sun and agents of oxidative stress like free radicals which are ubiquitous. This study aims to formulate an antioxidant cream based on Markhamia tomentosa ethanolic extract and Citrus sinensis oil. Seven cream formulations A1-A7 were prepared. Fusion technique was utilized in the formulation of the creams which were all water-in-oil emulsions. Formulations A1-A7 were characterized for pH, viscosity, organoleptic tests, spreadability, skin irritancy, antioxidant tests and centrifuge tests. The pH of the formulations A1-A7 was found to be in the range of 6.3 to 7.9. It was found that the creams were smooth, homogenous and formed an easily spreadable non-greasy film on the skin surface with no irritation on dermal application. Formulation A7 had the highest spreadability 116.7 ± 0.93 mm2g-1 while formulation A2 had the lowest spreadability 92.9 ± 0.10 mm2g-1. At a

concentration of 800 µg/mL the antioxidant activity of Markhamia tomentosa extract (75%) was close to that of Ascorbic acid (95%) which was the standard. Formulation A1 had the highest antioxidant activity while formulation A7 had the lowest due to the absence of M. tomentosa extract in its composition. All formulations passed the centrifuge test. A combination of the Markhamia tomentosa leaf extract and Citrus sinensis oil conferred a synergistic activity which led to potent antioxidant activity which is necessary for preventing oxidative stress and dermal photoaging. This herbal antioxidant formulation serves as a prototype that can be developed and translated to a marketable formulation for pharmaceutical application.

1.5 Formulation and Evaluation of Antioxidant Topical Cream Using Ethanol Extract of Markhamia Tomentosa Leaves

Background: Global warming which results from the thinning of the ozone layer has led to an increase in the level of exposure to ultraviolet rays from the sun. Long term exposure to these rays can lead to photo-aging. The aim of this study is to develop an antioxidant cream using ethanol extract of Markhamia tomentosa leaves as an active ingredient and to evaluate its physio-chemical properties as well as free radical scavenging and ferric reducing antioxidant activities.

Methods: The leaves of Markhamia tomentosa was obtained from Oke-Igbo, Ondo state, Nigeria in August 2019. The leaves of Markhamia tomentosa were dried, pulverized and macerated to obtain Markhamia tomentosa ethanol extract. Four water-in-oil emulsion creams containing Markhamia tomentosa ethanol leaf extract were formulated using fusion technique. Physico-chemical properties such as the pH, viscosity, skin irritancy, spreadability and organoleptic tests were evaluated. Subsequently the (1, 1-diphenyl1-2-picrylhydrazyl) DPPH radical scavenging and Ferric reducing antioxidant power assays were also carried out.

Results: All formulations were brown, with a smooth and non-greasy feel on dermal application. They had no characteristic smell and were easily washed off the skin. Formulations had a pH that fell withinthe range of 5.5-7.5 which is safe for dermal application. All formulations had a spreadability above 100 mm2 g -1 and exhibited non- Newtonian properties with a decrease in viscosity at the increase of shear stress emphasizing the ease of spreadability. FMT1 (Markhamia tomentosa ethanol leaf extract cream) had the highest antioxidant activity, which was concentration dependent, with a percentage radical scavenging activity of 76.48% and an absorbance of 0.276 in the ferric reducing antioxidant power assay, at wavelengths of 517 and 700nm respectively.

Conclusion: This Markhamia tomentosa ethanol leaf extract cream formulation exhibited strong antioxidant properties and can serve as a valuable archetype that can be developed and translated to a pharmaceutical antioxidant formulation for clinical use.

1.6 Topical application of Calendula officinalis (L.): Formulation and evaluation of hydrophilic cream with antioxidant activity[51]

The aim of the study was the formulation of suitable medicinal form for the topical application of Calendula officinalis L. Dry Calendula extract was used as an active compound. This extract was proved to be an effective scavenger of H2O2 radicals in in vitro studies with the mitochondria of rat cardiac muscles. Several compositions of the cream base were evaluated and the hydrophilic cream containing complex emulsifier was chosen as the delivery system. Subsequently, Calendula extract was incorporated, and the concentration of extract which provided significant antioxidant effect ($p < 0.05$), has been determined. Antioxidant activity of the cream with Calendula extract was due to the content of carotenoids, polyphenols and flavonoids. Cream with the best properties (0.9% of Calendula extract) contained 0.73 ± 0.04 mg/100 g of total carotenoids expressed as - carotene. This cream was then examined microscopically, and stability studies including evaluation of organoleptic properties, microbiologic quality, and determination of variations in total carotenoid content during the storage were made. Achieved results suggest that developed cream with Calendula extract poses the good quality emulsion system warranting the stability of carotenoids, and thus the therapeutic, namely antioxidant activity of preparation. Key words: Calendula officinalis, dry extract, antioxidant activity, hydrophilic cream, preparation, evaluation.

1.7 Characterization of cosmetic cream with *Mesembryanthemum crystallinum* plant extract: influence of formulation composition on physical stability and antioxidant activity[52]

Cosmetic or pharmaceutical composition containing superoxide dismutase (SOD) was usually used in topical administration, particularly, in fighting against skin ageing and in the protection of the skin against radiation exposure. Mesembryanthe mum crystallinum is a halophyte plant widely used in the traditional medicine, characterized by the presence of antioxidants enzymes in responses to abiotic stresses. In the present study, we prepared a formulation with M. crystallinum extract characterized by naturally occur ring SOD and catalase in association with other antioxidants molecules. The SOD activity was measured by 3-(4,5-dimethyldiazol-2-yl)-2,5 diphenyl-tetrazolium bromide/riboflavin method, catalase by colorimetric method and the total anti-radical activity was measured by 1,1- diphe nyl-2-picrylhydrazyl radical (DPPH) method. Formulations contain a significant SOD activity (8.33 U mg)1), a catalase activity (0.5 · 107 UC) and an anti-radical activity (30% of DPPH inhibition). The formulation storage (15 days at 4C) showed a marked loss of total antioxidant capacity. The addition of the M. crystallinum extract induced also a reduction in formulation viscosity and pH.

1.8 In vitro evaluation of Spirulina platensis extract incorporated skin cream with its wound healing and antioxidant activities

Context: Algae have gained importance in cosmeceutical product development due to their beneficial effects on skin health and therapeutical value with bioactive compounds. Spirulina platensis Parachas (Phormidiaceae) is renowned as a potential source of high- value chemicals and recently used in skincare products.

Objective: This study develops and evaluates skin creams incorporated with bioactive S. platensis extract. Materials and methods: Spirulina platensis was cultivated, the aqueous crude extract was prepared and in vitro cytotoxicity of S. platensis extract in the range of 0.001–1% concentrations for 1, 3 and 7d on HS2 keratinocyte cells was determined. Crude extracts were incorporated in skin cream formulation at 0.01% (w/w) concentration and in vitro wound healing, and genotoxicity studies were performed. Immunohistochemical staining was performed to determine the collagen activity.

Results: 0.1% S. platensis extract exhibited higher proliferation activity compared with the control group with 198% of cell viability after 3d. Skin cream including 1.125% S. platensis crude extract showed enhanced wound healing effect on HS2 keratinocyte cell line and the highest HS2 cell viability % was obtained with this concentration. The micronucleus (MN) assay results indicated that S. platensis extract incorporated creams had no genotoxic effect on human peripheral blood cells. Immunohistochemical ana lysis showed that collagen 1 immunoreactivity was improved by increased extract concentration and it was strongly positive in cells treated with 1.125% extract incorporated skin cream.

Conclusions: The cell viability, wound healing activity and genotoxicity results showed that S. platensis incorporated skin cream could be of potential value in cosmeceutical and biomedical applications.

1.9 In vitro and in vivo evaluation of efficacy and safety of photoprotective formulations containing antioxidant extracts[53]

Chronic exposure to solar radiation could contribute to premature skin aging and skin cancer. Skin presents its own antioxidant defence, however when defences are out of balance, reactive oxygen species could damage biological structures. In the present work, an oil-in-water photoprotective emulsion was developed and Bauhinia microstachya var. massambabensis Vaz, Fabaceae, extracts at 1% (obtained by extraction with different solvents) were added to this emulsion. In vitro and in vivo efficacy and safety of the formulations were evaluated. Spectrophotometric methods and in vivo Colipa test were performed to evaluated efficacy of the formulations, through sun protection factor (SPF) determination and UVA protection factor assessment. To the in vitro safety assessment HET-CAM, CAM-TBS and Red Blood Cell tests were performed. Results showed that both extracts contributed to a higher in vivo photoprotection (SPF 18) when compared to the formulation without extract (SPF 13), this result could be attributed to the antioxidant activity of the plant extracts that act by capturing reactive oxygen species. Concerning safety, all formulations were considered non-irritant according to in vitro tests. Formulations containing extracts could be considered efficient and safe for cosmetic use since they presented higher sun protection factor and passed the toxicity tests.

1.10 Dittrichia viscosa L. Ethanolic Extract Based Ointment with Antiradical, Antioxidant, and Healing Wound Activities[54]

Dittrichia viscosa which belongs to the Asteraceae family is frequently used to treat hematomas and skin disorders in Mediterranean herbal medicine. This study aims to validate its antioxidant effects and its potential on healing wounds. The ethanolic extract of D. viscosa leaves was formulated as 2.5% and 5% (w/w) in ointment bases on the beeswax and sesame oil. During this study, the ethanolic D. viscosa extract, ointments containing 2.5% and 5% of D. viscosa extract, and the vehiculum were assessed for their total phenol content (TPC), caffeoylquinic acid content (CQC), and antioxidant activities using complementary methods (TAC, the DPPH, ABTS, FRAP, and the BCB). The effects on wound healing of obtained ointments were evaluated by excision of the wound in amice model for 12days. Subsequently, the excised wound areas were measured at the 3rd, 9th, and 12th days. The skin tissues were isolated for histological studies. The ointments containing D. viscosa extract (2.5%, 5%) possessed a considerable TPC, CQC, radical scavenging potential, and antioxidant activities compared to the vehiculum. Treated animals with ointments with extract from D. viscosa at 2.5%and5% showed almost and totally healed wounds compared to the vehiculum and control groups, evidenced by good skin regeneration and reepithelialization. The present work showed the role of D. viscosa antioxidants exerted by its polyphenolic compounds, particularly caffeoylquinic acids, in enhancing wound healing.

1.11 Assessment of Rose Water and Evaluation of Antioxidant and Anti-inflammatory Properties of a Rose Water Based Cream Formulation[55]

Photo-aging is a universal dilemma that occurs in our population due to constant contact with ultraviolet radiation. The utilization of antioxidants is a successful approach to avoid symptoms associated to the photo-induced aging of the skin. In view of this, present study was designed to prepare and evaluate the antioxidant & anti-inflammatory activity of cream comprising the aqueous petals extract of Rosa damascena for its radical scavenging and protein denaturation activity. Antioxidant activity assessed using standard ascorbic acid (ferric reducing power assay), and anti-inflammatory

activity assessed using standard diclofenac sodium measuring of the %age inhibition of protein denaturation. The rose water contains the major phytoconstituents which are polyphenolic compounds flavonoids, tannins, triterpenoids, saponins which are mainly responsible for the antioxidant and anti- inflammatory properties. Out of three cream formulations (F1, F2, and F3). F1 cream formulation showed the highest antioxidant (81.55%) and anti-inflammatory activity (80.6%) at 1000µg/ml. the result noted to be concentration dependent. The IC 50 value of F1 formulation cream was 257.39 while for F2 cream formulation 374.41. The present results indicate that the Rosa damascena petals extract (Rose water) has a good potential for cosmetic product development.

1.12 Formulation and evaluation of antibacterial and antioxidant polyherbal lotion[56]

There is increased scientific evidence that plants possess a vast and complex arsenal of active ingredients which have the ability to calm or smooth the skin as well as restore actively, heal and protect the skin. The present work deals with the development and evaluation of the poly herbal lotion containing Trigonella foenum-graecum, Citrus lemon, Matricaria chamomilla and Cymbopogon citrates. Different types of formulations oil in water (O/W) herbal lotions namely F1 to F13 were formulated by incorporating different concentrations of stearic acid and Triethanolamine. Formulation of Triethanolamine and stearic acid was optimized as 2.52:9.35. The prepared lotion was evaluated for its antimicrobial, antioxidant and pharmaceutical parameters. The lotion formulation showed no redness, edema, inflammation and irritation during sensitivity test indication that it is safe to use. Stability studies of the lotion showed that the lotion was stable after three months.

Key Words: Herbal formulation, Triethanolamine, Stearic acid, Antioxidant, Antibacterial.

1.13 Topical Formulations of Butterfly Pea Flower (Clitoria ternatea L.) Ethanol Extract by Ultrasound assisted Extraction: Formulation and Antioxidant Activity Evaluation[57]

Abstract The antioxidant action of Butterfly pea flower (Clitoria ternatea L.) is believed to be attributed to its flavonoids, alkaloids and polyphenols components. These chemicals function as antioxidants, effectively neutralising free radicals and providing protection against skin damage caused by UV radiation. The objective of this study was to ascertain the IC50 value of butterfly pea extract and thereafter to evaluate the degree of customer preference and convenience towards pharmaceutical items. This study utilised ultrasound-assisted extraction with a solvent technique employing 96% ethanol to generate extracts. These extracts were then compounded into several topical formulations including cream, gel and face spray. Initial assessments were conducted on all compositions, encompassing organoleptic, homogeneity, pH, viscosity, specific gravity, adhesion, spreadability, wetness and irritation.

This study also incorporated acceptance and customer preference as factors. The antioxidant activity of BPF extract was determined using the 2,2-diphenyl-1 picrylhydrazyl (DPPH) technique by estimating the IC50 value. The analysis revealed that the BPF extract had a yield of 38.75%. The IC50 values for the gel, cream and face mist preparations were 97.70, 49.14 and 73.89 ppm respectively. The cream recipe had the highest level of antioxidant activity. The determination of consumer preference has indicated that the majority of consumers favours face mist over other formulations.

Keywords: Antioxidants, butterfly pea flower, pharmaceutical preparations and ultrasound-assisted extraction.

1.14 Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formulations containing Calendula officinalis L. leaf extract[58]

Calendula is used widely in cosmetic formulations that present phenolic compounds in their chemical constitution. The objective of our research was to develop and evaluate the stability of topical formulations containing 5% hydro-ethanolic extract of calendula leaves, including spreadability, and in vitro photo protective, and antioxidant capacity. To evaluate the stability, we used organoleptic characteristics, pH, and viscosity parameters. Antioxidant capacity was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, and the photo-protective capacity by SPF spectrophotometric measure. All formulations were stable. The calendula extract formulations in gel and cream showed no significant variations in pH, and the cream formulations presented lower viscosity variations than gel formulations. The spreadability of the gel formulations was superior to those in cream. The formulations also presented good antioxidant capacities and an FPS of around 1.75. In accordance with the results, the formulations can be used as antioxidants, but considering the low SPF obtained, calendula cannot be considered as a stand-alone sunscreen yet may well be tested in future studies towards verifying enhancement of synthetic sunscreens.

1.15 Nutritional value, chemical composition, antioxidant activity and enrichment of cream cheese with chestnut mushroom Agrocybe aegerita (Brig.) Sing[59]

A very well-known and appreciated mushroom, Agrocybe aegerita (Brig.) Sing, was the subject of chemical profiling, antioxidant assays and sensory evaluation test in cream cheese. Methanolic extract obtained from a wild sample of A. aegerita fruiting body was fully chemically identified. Sample was found to be rich in carbohydrates (84.51 g/ 100 g dw), ash and proteins (6.69 g/100 g dw and 6.68 g/ 100 g dw, respectively). Trehalose was the main free sugar while

malic acid was the most abundant organic acid. Four isoforms of tocopherols were identified; γ- tocopherol was the dominant isoform with 86.08 µg/100 g dw, followed by β tocopherol, δ-tocopherol and α-tocopherol (8.80 µg/ 100 g dw, 3.40 µg/100 g dw and 2.10 µg/100 g dw, respectively). Polyunsaturated fatty acids were predominant, with linoleic acid as the most prominent one (78.40 %). Methanolic extract of chestnut mushroom exhibited high antioxidant activity. Sensory evaluation test included grading by panelists and comparing the overall acceptability of cream cheese alone and enriched cream cheese with dry powder of A. aegerita. General conclusion of the participants was that the newly developed product was more like able in comparison to cream cheese alone. Due to the health-beneficial effects of antioxidants and wealth of chemically identified nutrients, A. aegerita is a promising starting material for incorporation on larger scale products.

Keywords: Agrocybeaegerita. Chemical profile. Antioxidant potential. Cream cheese. Sensory evaluation test.

2. CONCLUSION

The current study was conducted to develop and test a herbal antibiotic cream combining ethanolic extracts of various medicinal plants with antibacterial activity. The study found that ethanolic extraction is an effective method for extracting biologically active phytoconstituents such alkaloids, flavonoids, glycosides, tannins, phenolic compounds, terpenoids, and saponins from plant materials. These phytochemicals are primarily responsible for the antibacterial and wound healing activities of herbal products.

Different cream formulations were created by combining various ethanolic plant extracts with appropriate cream bases. The formulations were tested for a variety of physicochemical and pharmacological criteria, including appearance, color, odor, texture, consistency, pH, homogeneity, spreadability, viscosity, washability, extrudability, irritancy, and stability. The evaluation results showed that all formulations had acceptable physical properties and were appropriate for topical application. During storage, the creams demonstrated a smooth texture, good consistency, easy spreadability, and no indications of phase separation or grittiness.

The formulations' pH was found to be within the permitted range of skin pH, indicating that the creams are safe and non-irritating for dermal use. Stability experiments demonstrated that the formulations were stable under various storage settings, with no notable changes in color, odor, consistency, or antibacterial activity. The absence of skin irritation and redness demonstrated the safety of the herbal cream formulations.

The antimicrobial testing revealed that the cream formulations had strong antibacterial activity against some pathogenic germs. Among the generated formulations, the optimal formulation incorporating mixed plant extracts demonstrated higher antibacterial activity due to the synergistic impact of phytoconstituents found in various plants. The herbal cream exhibited a zone of inhibition comparable to that of standard antibiotic preparations, indicating that it has the potential to cure bacterial skin infections.

The study also demonstrated the benefits of herbal formulations over synthetic antibiotic creams. Herbal creams are often associated with less adverse effects, lower risk of germ resistance, improved patient compliance, simple access to raw materials, and economic effectiveness. In addition to antibacterial activity, plant extracts contain natural antioxidants and anti-inflammatory substances, which may help with wound healing and skin protection.

From the overall results, it can be concluded that the formulated ethanolic extract-based herbal antibiotic cream is a promising topical preparation with good pharmaceutical properties and effective antibacterial activity. The use of medicinal plant extracts in topical formulations provides an alternative approach for the management of minor skin infections and related disorders. However, further studies including detailed pharmacological investigations, toxicity studies, long-term stability testing, and clinical trials are necessary to confirm the therapeutic efficacy, safety, and commercial applicability of the developed formulation.

Thus, the present work supports the growing interest in herbal medicine and demonstrates the potential of medicinal plant extracts in the development of safe, effective, and economical topical antibiotic formulations for pharmaceutical and healthcare applications.

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