Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.4206

# FORMULATION OF POLYHERBAL CREAM BASED ON PRESENCE OF SECONDARY METABOLITES, ITS ORGANOLEPTIC ASSESSMENT AND THERAPEUTIC EFFECT ON DERMATITIS PATIENTS

**Shahlla Umbreen[1], Farheen Ansari[2*]**

[1,2*]Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan

***Corresponding Author:** Farheen Ansari
*Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan
Email: farheen.ansari@imbb.uol.edu.pk

**Abstract**

**Background and Objective:** Dermatitis is a multifactorial skin inflammatory diseases and economic burden due to high cost treatment. This disease decreases the quality of life. The objective of this research is to formulate a local applicant by using plant extracts and to evaluate its effects clinically on patients.

**Material and Methods:** The plant materials, leaves of *Lawsonia inermis*, fresh bark of *Ficus carica, Carica papaya* and *Pisidium guajava* were collected, extracts prepared, phytochemicals analyzed and cream is prepared by mixing oil phase and aqueous phase. The organoleptic evaluation of cream was done and then by designing the study the cream applied disease patients.

**Results**: Results was measured after 4 weeks of application of cream and clinical parameters was selected before the study and productive change measured in these like extent area, redness, swelling, crusting, itching. Then SEM of SCORAD values measured which shows the remarkable curing effects.

**Conclusion:** The plants used in the formulation of cream was found to be promising for the treatment of dermatitis. On the base of results, cream was effective in minimalizing the symptoms like itching, inflammation, skin thickening and dryness. It will improve the quality of life of dermatitis patients.

**Keywords:** Polyherbal Cream, Phytochemical Analysis, Skin Infections, Dermatitis,

## Introduction

Atopic Dermatitis is an inflammatory disease of skin in response to small amount of environmental proteins like food allergen, dust mites and pollens that leads to cutaneous hyperactivity. It is chronic, relapsing condition and eczema is the acute manifestation of this disease (Thomsen, 2014). Its incidence is high not only in Urban countries but also in developing ones (Sroka and Trzeciak 2021) and affects one fifth people in their life time with great variation of pervasiveness (Asher *et al*., 2006). *Pathogenesis*: It is multifactorial disease caused by immune dysregulation, defect in epidermal barrier, disruption of skin's microbial balance and environmental factors that disrupts the epidermis

and proceeds to pruritic skin (Frazier et al., 2020). Ascribable to the disturbance in gene that encodes proteins of immune system and of epidermis chiefly Fillagrin, thus causes epidermal barrier loss. The fillagrin monomers builds the stratum corneum by catalytic activity and mutation in this protein gene leads to increase in trans-epidermal water loss, skin dryness, high pH, disequilibrium in ceramides, triglycerides and free fatty acids. Mutation in genes encoding claudins and occludins which are the proteins of intercellular junctions causes connection degradation, increase permeability, penetration of antigens and incentive to cytokines (Sroka and Trzeciak 2021).

The surface of epidermis inhabits the microbial flora (Segre *et al*., 2019), the quality and quantity of flora varies according to individual and location on human body. This variation depends on skin thickness, temperature, humidity, sebum content, pH, folds of body and exposure to UV light (Dréno *et al*., 2016). In case of dermatitis, there is a decrease in the normal flora members like *Cutibacterium*, *Corynebacterium*, *Streptococcus* while there is an increase in *Staphylococcus* species, chiefly the *Staphylococcus aureus*. The factors like higher skin pH, decrease fillagrin, disfigured corneocytes, and less amount of antimicrobial peptides favors the growth of *Staphylococcus aureus* (Paller *et al*., 2019) which in turn, release and stimulate proteases e.g. metalloproteinases, keratinocyte serine proteases to further dissolve the corneum. Subsequently, destroy the epidermal barrier and increase the permeability predisposing to inflammatory response (Nakatsuji and Gallo 2019).

If not treated on time, infections spreads from top layer of skin to follicles, deeper layers and results in folliculitis, cellulitis and lyme disease (Tabassum and Hamdani 2014).

*Clinical features*: The symptoms of this disease includes itching, skin eruptions, redness, papules, oozing lesions, various degree of dryness and skin lichenification (Avena et al., 2017). These symptoms decrease the quality of life, mainly the persistent itch causes sleep problems, insomnia, avoid social interactions and also affects the daily activities (Silverberg *et al*., 2018). If not treated on time, then patient more likely develop urinary tract infections, pharyngitis, ear infections, asthma and hay fever (Frazier 2020). The fact is that the disease involves the whole family and has long course treatment thus arises as a social problem by escalation in economic burden (Xu *et al*., 2019).

*Therapeutic intervention*: Dermatitis has extensive clinical phenotype reflecting multidirectional component interactions including environmental, microbial, epidermal barrier, inflammatory and immune responses and itch scratch cycle which in turn helps to understand the therapeutic and preventive interventions (Bieber 2022).

To cure skin diseases, the basic commercial formulations includes poultice, compress, decoction, cream and ointments and the best therapeutic potential explored from medicinal plants (Tsioutsiou *et al*., 2022). According to World Health organization, more than eighty percent depends on herbal medicine with aspect to primary health care due to less side effects and best therapeutic effects in perspective of antimicrobial resistance. The drug delivery through skin is a non-invasive way and has been an auspicious concept owing to the fact of ease access, greater exposure to blood and lymphatics and immense surface area. Herbal ointments are combination of plant powder dissolved and emulsified in the base which can be either anhydrous and sometimes oleaginous in nature with water absorbing or removal capability (Awad *et al*., 2015).

The objective of this research was to prepare and evaluate an ointment from the extracts of plants that has been used for the treatment of skin diseases since ancient times. The plants are the rich source of secondary metabolites which are known for great therapeutic potential for example antioxidant, antibiotic, anticancer, anti-inflammatory, skin regenerative and many others. On that basis, plants with maximum amount of flavonoids and phenolic acids that has great absorption capacity through skin are selected to prepare an ointment.

*Medicinal Plants*: *Lawsonia inermis* belongs to family lythraceae referred as henna has been traditionally used for the treatment of liver and digestives diseases, ulcers, tissue loss in leporosy and also acknowledged for antibacterial, antiparasitic, analgesic, anti-inflammatory, immune modulatory, antitrypanosomal and antioxidant actions (Badoni *et al*., 2014). Ancient scientists like Rhazes, Avicenna, and Aghili, reported the marvelous results of henna as antimicrobial and skin enhancing ingredient (Niazi, Mehrabani *et al*., 2020).

The bark of *Ficus carica*, belongs to family Moraceae known as anjeer or fig, has febrifuge, antiseptic and vermicidal properties. Its decoction is used to treat skin ulcers and diabetes (Mir *et al*., 2023). *Carica papaya* belongs to family Caricaceae known as papaya, has great importance due to the presence of laticifers in all of its parts. It embraces many pharmacological properties like antitumor, anti-inflammatory, antihypertensive and wound healing, chiefly anti allergic and used in sports injuries due to presence of papain enzyme (Fatima and Shahid 2018). It acts as antioxidant by neutralizing the free radicals due to vitamins (A, B, E and C), minerals (Mg, K) and folate (Vij and Prashar 2015). *Pisidium guajava* belongs to family Myrtacea, also known as guava, traditionally used as cough sedative (Joseph and Priya 2011). It has the ability to control diabetes, obesity, hypertension and inhibit growth of Staphylococcus species consequently treats acne, rashes and ringworm due to the presence of guajaverin and psidiolic acid (Growther 2018).
The aim was to formulate a medicine effective as local applicant for the patients of dermatitis.

## Material and Methods

### *Collection of plant material*

The fresh leaves of *Lawsonia inermis*, fresh bark of *Ficus carica, Carica papaya* and *Pisidium guajava* were collected from Iqbal town, Lahore Pakistan. Plants were identified and authenticated by Prof. Abdul Rehman Nizai, Botany Department, Punjab University, Lahore Pakistan and Voucher No. were issued as LAH#10922 for *Lawsonia inermis*, LAH#08922 for *Ficus carica*, LAH#09922 for *Carica papaya* and LAH#60922 for *Pisidium guajava.*

### *Preparation of Extracts*

The collected plant parts were washed and dried at room temperature and then grinded to a fine powder. The dried powder was extracted using soxhlet extractor with different solvents as ethanol for *Lawsonia inermis* leaves and *Pisidium guajava* bark whereas Acetone for *Ficus carica* and *Carica papaya* bark. Concentrated extract was collected, filtered and the evaporated to dryness until dry mass is obtained. (Matangi, Santosh *et al*., 2014). Weight of extract was recorded and yield of extract was calculated.

### *Determination of phytochemical constituents*

Preliminary phytochemical analysis was done by using standard qualitative methods. The qualitative analysis is done by GCMS techniques whereas for the quantification of flavonoids and phenolic compounds were determined by High Performance Liquid Chromatographic (HPLC) technique (Awad *et al*., 2015).

### *Formulation of cream*

For the formulation of polyherbal cream, slab method was followed (Chavan *et al*., 2020). In first step, oil phase was prepared and for this the stearic acid an emulsifier, cetyl acohol and beeswax were taken in the beaker and heated on water bath for uniform mixing. The preservatives and water soluble ingredients like Methayl paraben, propyl paraben, triethanolamine, white soft paraffin and 3g powder extracts of *Lawsonia inermis* leaves and bark of *Ficus carica, Carica papaya* and *Pisidium guajava* were dissolved in distilled water and heated at 70℃ to obtained the aqueous phase. The aqueous phase was added in small portions to the oil phase with continuous stirring until a smooth semisolid mass is obtained. The formed cream was further evaluated.
Oil phase= Stearic acid. Cetyl alcohol, beeswax Aqueous phase= Methayl paraben, propyl paraben, triethanolamine, white soft paraffin, Sodium metabisulphite, extract powders, menthol

### *Organoleptic evaluation of Cream*

The above formulated cream was subjected to evaluation for color, odor, consistency by rubbing cream on hand manually, Homogeneity, pH for this cream solution was prepared in 10ml distilled water and then set aside for 2 hours and the pH measure with digital pH meter, spread ability checked

by placing cream in between two slides a definite weight placed to compress for uniform thickness and after specified time spreadability was calculated by formula, wash ability was checked by applying cream on skin and then ease extends of washing with water, and irritancy test checked by applying cream on dorsal skin of left hand and site observed for 24 to 28 hours (Awad *et al*., 2015).

***Evaluation of cream effectiveness on dermatitis patients***

For this study, approval was taken from the departmental Bioethics, Biosafety and Biosecurity Committee (BBBC) of The University of Lahore, Pakistan and the number was allotted (Ref. IMBB/UoL/21/1037). For this Helsinki's Declaration was strictly followed. 25 volunteer dermatitis patients (lesion at arm, cheek, leg, feet, hand and back etc.) reported at Family Natural dawakhana, Township Lahore, Pakistan and 25 volunteers in Placebo group were selected, whose age ranges from 25 to 40 years of either sex.

Infection sizes was measured, ranges from 105 to 500mm$^{2.}$ On first day, Patch test was performed to determine any sign of reaction of cream of forearm of each member of study group. After 48 hours only the base (B Cream) to the control group and cream with active ingredient (D cream) to the D group (Dermatitis group) was given to apply for 4 weeks twice daily (Akhtar 2011).

***Study Design***

A single blind study was performed. Two formulations named B cream (base cream) was given to control group and D cream (active ingredients) given to the disease group with instructions of application and the results were measured.

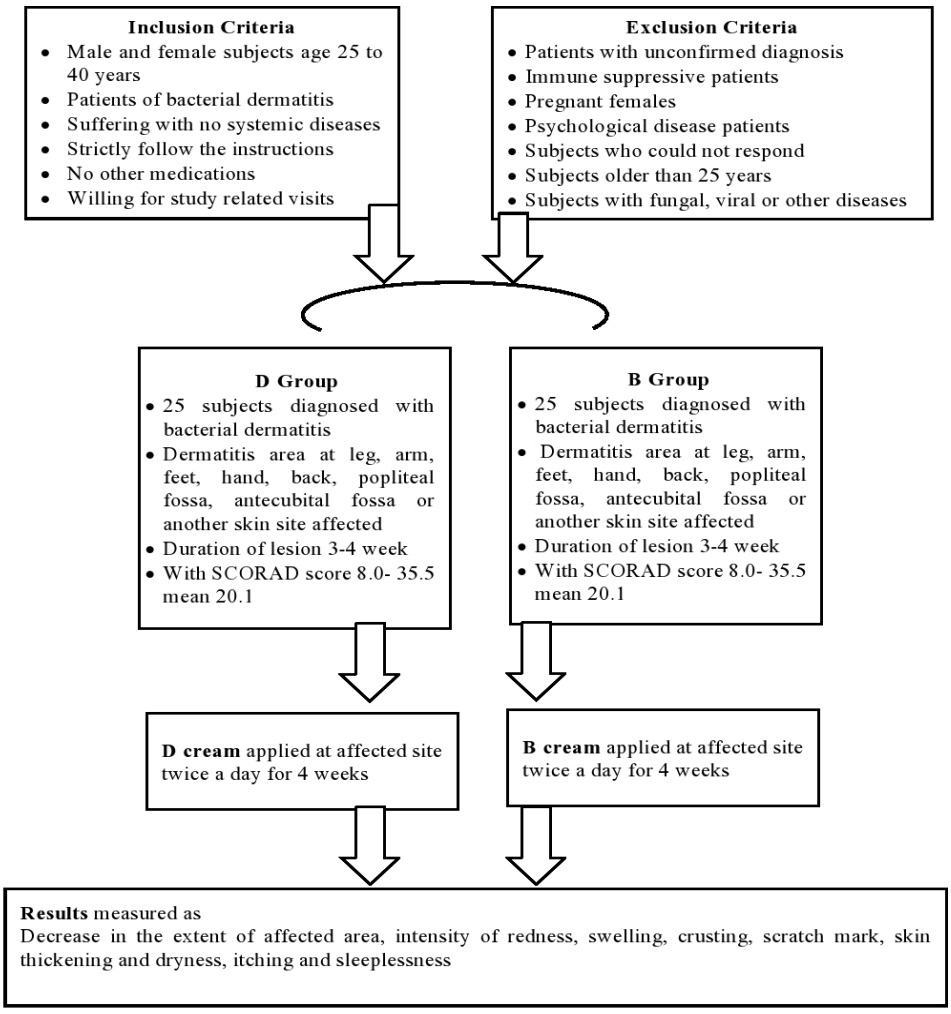


**Fig 1:** Study Design to evaluate the effectiveness of Cream

***Statistical Analysis***

The data expressed as mean ± SEM of SCORAD (Objective and Subjective) score measured with confidence interval of 95%.

## Results

***Preparation of Extracts and Yield Percentage***

By using different plants part as leaves of *Lawsonia inermis*, fresh bark of *Ficus carica*, *carica papaya* and *Pisidium guajava*, extracts were prepared and color and texture of extract recorded as depicted in supplementary table 1. Then the percentage yield calculated and displayed in Table 1 which indicates the maximum percentage yield obtained from *Lawsonia inermis* leaves and *Carica papaya* bark of 44 %.

**Supplementary Table 1: Morphology of plant extracts**

| Extract Code | Color | Texture | Image |
|---|---|---|---|
| FCBA | Orange brown (Like brown sugar) | Sticky Powder |  |
| LILE | Dark brown | Gum like |  |
| CPBA | Marmalade orange | Powder |   |
| PGBE | Peanut brown | Powder |   |

FCBA-Ficus carica Bark Acteone, LILE- Lawsonia inermis Leaves Ethanol, CPBA- Carica papaya Bark Acetone, PGBE- Pisidum guajava Leaves Ethanol

**Table 1: Percentage yield of Plant extracts**

| Plant Name | Plant Part (25 g) | Solvent (300ml) | |
|---|---|---|---|
| | | Extract Quantity (g) | Extract Yield (%) |
| ***Ficus carica*** | Bark | 5 | 20 |
| ***Lawsonia inermis*** | Leaves | 11 | 44 |
| ***Carica papaya*** | Bark | 11 | 44 |
| ***Psidium guajava*** | Bark | 7.9 | 31.6 |

***Preliminary, Qualitative and Quantitative phytochemical analysis***

The results measured for preliminary phytochemical analysis displayed in Table 2. Different standard tests performed and observed that FCBA and PGBE was found positive for 9 phytochemicals whereas LILE and CPBA was positive for 7 out of 10 phytochemical tests.

**Table 2: Preliminary Phytochemical Analysis**

| Compounds | FCBA | LILE | CPBA | PGBE |
|---|---|---|---|---|
| **Carbohydrates** | + | - | + | - |
| **Alkaloids** | + | - | + | - |
| **Tannins** | + | + | - | + |
| **Glycosides** | + | - | + | + |
| **Flavonoids** | + | + | + | + |
| **Phenols** | + | + | + | + |
| **Diterpenes** | + | - | + | + |
| **Saponins** | + | + | - | + |
| **Quinones** | - | + | - | + |
| **Steroids** | - | + | - | + |
| **Resins** | + | + | + | + |
| **Total (11)** | **9** | **7** | **7** | **9** |

'-' Absence, '+' Presence of compound, FCBA- *Ficus carica* Bark Acetone, LILE- *Lawsonia inermis* Leaves Ethanol, CPBA- *Carica papaya* Bark Acetone, PGBE- *Psidium guajava* Bark Ethanol On GCMS analysis results demonstrated in Table 3, *Ficus carica* Bark Acetone (FCBA) extract give a picture of 14 compounds with maximum area percentage of 40.79 % by 1,2,3-Benzenetriol, while minimum of 0.16 % by 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one. *Lawsonia inermis* Leaves Ethanol (LILE) extract was found to be the source of 18 compounds. The maximum area percentage was covered by 1,2,3-Benzenetriol as 67.36 % and of minimum covered by Cyclopentanone, dimethylhydrazone of only 0.5%. *Carica papaya* Bark Acetone (CPBA) extract was full of compounds (26) and the maximum area percentage was allocated to 1-Isobutyl-7,7-dimethyl-octahydrisobenzofuran-3a-ol of 10.77% while that of minimum was given to Benzoic acid at 0.21%. *Psidium guajava* Bark Ethanol (PGBE) extract showed the presence of 17 compounds and maximum area percentage was covered by Phthalic acid, di(2-propylpentyl) ester which was 18.36 % while minimum was of 2(1H)-Naphthalenone, octahydro-4 measured 0.68%.

**Table 3: Qualitative analysis of Plant Extracts by GCMS**

| Compound Name | Synonym | Retention Time/Min | Area % | Molecular Formula | Molecular Weight (g/mol) | Plant Extract |
|---|---|---|---|---|---|---|
| 1,2,3-Benzenetriol | Pyrogallol | 9.114 | 40.79 | $C_6H_6O_3$ | 126.11 | FCBA,CPBA |
| Propyleneglycol monoleate | 2-Hydroxypropyl oleate | 24.706 | 18.28 | $C_{21}H_{40}O_3$ | 340.5 | FCBA |
| Oleic Acid | 9-(Z)-octadecenoic acid | 17.24 | 10.06 | $C_{18}H_{34}O_2$ | 282.4614 | FCBA, LILE, CPBA,PGBE |
| Phthalic acid, di(2-propylpentyl) ester | | 20.452 | 2.71 | $C_{24}H_{38}O_4$ | 390.5561 | FCBA, LILE, PGBE |
| n-Hexadecanoic acid | Palmitic acid | 15.623 | 2.28 | $C_{16}H_{32}O_2$ | 256.4241 | FCBA, LILE, CPBA,PGBE |
| Octadecanoic acid | Stearic acid | 17.475 | 2.22 | $C_{18}H_{36}O_2$ | 284.4772 | FCBA, LILE, CPBA,PGBE |
| Catechol | 1,2-Benzenediol | 6.79 | 2.02 | $C_6H_6O_2$ | 110.1106 | FCBA, LILE |
| tert-Butyl(2-isopropyl-5-methylphenoxy)dimethylsilane | Thymol | 27.049 | 1.36 | $C_{16}H_{28}OSi$ | 264.478 | FCBA, LILE |
| 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | Glyceryl Monooleate | 21.514 | 0.94 | $C_{21}H_{40}O_4$ | 356.5399 | FCBA |
| Glycerol 1-palmitate | α-Monopalmitin | 20.172 | 0.91 | $C_{19}H_{38}O_4$ | 330.5026 | FCBA, LILE, PGBE |
| 2-[(tert-butyldimethylsilyl) oxy]-Benzene | Pyrogallol, 3TBDMS derivative | 26.681 | 0.65 | $C_{24}H_{48}O_3Si_3$ | 468.9 | FCBA, LILE |
| Phenol | Carbolic acid | 3.794 | 0.45 | $C_6H_6O$ | 94.1112 | FCBA |
| Methyl 12-oxo-9-dodecenoate | Methyl (9E)-12-oxo-9-dodecenoate | 21.722 | 0.44 | $C_{13}H_{22}O_3$ | 226.312 | FCBA |
| 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one | 3,5-Dihydroxy-2,3-dihydro-6-methyl-4-pyran-4-one, dihydroxy maltol | 5.761 | 0.16 | $C_6H_8O_4$ | 144.1253 | FCBA, LILE, CPBA,PGBE |
| 1,2,3-Benzenetriol | Catechol (phenol) | 9.148 | 67.36 | $C_6H_6O_2$ | 110.1106 | LILE, CPBA |

| | | | | | | |
|---|---|---|---|---|---|---|
| trans-Cinnamic acid | (E)-3-Phenyl-2-propenoic acid | 9.616 min | 5.68 | $C_9H_8O_2$ | 148.1586 | LILE |
| 1,2Bis(trimethylsilyl)benzene | Trimethyl[4-(trimethylsilyl)phenyl]silane | 24.714 | 5.38 | $C_{12}H_{22}Si_2$ | 222.4741 | LILE |
| (Z,Z)-9,12-Octadecadienoic acid | Linoleic acid | 17.169 | 1.64 | $C_{18}H_{32}O_2$ | 280.4455 | LILE,CPBA, PGBE |
| 5-Hydroxymethylfurfural | 5-(hydroxymethyl) furan-2-carbaldehyde | 6.729 | 1.15 | $C_6H_6O_3$ | 126.11 | LILE, CPBA,PGBE |
| 4-amino-2-(ethylthio)-5-Pyrimidinecarboxylic acid | 2-(5-ethyl-2-imino-1,3,4-thiadiazin-6-yl)2-hydroxyacetaldehyde | 12.612 | 0.77 | $C_7H_9N_3O_2S$ | 199.23 | LILE |
| Dibutyl phthalate | Palatinol C | 15.36 | 0.53 | $C_{16}H_{22}O_4$ | 278.3435 | LILE |
| 1,2:5,6-bis-O-(1-methylethyldiene)-D-Mannitol | Diisopropylidene mannitol | 11.959 | 0.48 | $C_{12}H_{22}O_6$ | 262.2995 | LILE |
| Octadecanoic acid, 2-hydroxy-1-(hydroxyl-methyl)ethyl ester | Stearic acid β-monoglyceride | 21.728 | 0.45 | $C_{21}H_{42}O_4$ | 358.5558 | LILE |
| trans-1-Ethyl-4-Methylcyclohexane | 1-Methyl-trans-4-ethylcyclohexane | 21.518 | 0.7 | $C_9H_{18}$ | 126.2392 | LILE |
| Cyclopentanone, dimethylhydrazone | Cyclopentanon-N,N-dimethylhydrazone | 5.097 | 0.5 | $C_7H_{14}N_2$ | 126.2 | LILE |
| 1-Isobutyl-7,7-dimethyl-octahydrisobenzofuran-3a-ol | 1-Isobutyl-7,7-dimethylhexahydro-2-benzofuran-3a(3H)-ol | 12.433 | 10.77 | $C_{14}H_{26}O_2$ | 226.35 | CPBA |
| Dimethyl 2,5-thiophenedicarboxylate | thiophene-2,5-dicarboxylic acid dimethyl ester | 12.753 | 8.71 | $C_8H_8O_4S$ | 200.21 | CPBA |
| Butanoic acid, propyl ester | Butyric acid, propyl ester | 7.439 | 4.83 | $C_7H_{14}O_2$ | 130.1849 | CPBA |
| Glycerin | Trihydroxypropa-ne | 4.432 | 2 | $C_3H_8O_3$ | 92.0938 | CPBA |
| 2-Propoxy-succinic acid, dimethyl ester | Dimethyl 2-propoxysuccinate | 7.524 | 1.77 | $C_9H_{16}O_5$ | 204.22 | CPBA |
| N-Methyl-1-noradamantane carboxamide | | 11.286 | 1.16 | $C_{11}H_{17}NO$ | 179.26 | CPBA |
| 4-Hydroxy-3,5-dimethoxybenzoic acid | Syringic acid | 4.555 | 0.99 | $C_9H_{10}O_5$ | 198.1727 | CPBA |
| DL-Proline, 5-oxo-, methyl ester | Methyl 5-oxoprolinate | 8.866 | 0.78 | $C_6H_9NO_3$ | 143.1406 | CPBA |
| 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl | 5-Hydroxymaltol | 6.436 | 0.75 | $C_6H_6O_4$ | 142.1094 | CPBA |
| 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester | Oleic acid, 2-hydroxyethyl ester | 21.531 | 0.59 | $C_{20}H_{38}O_3$ | 326.5139 | CPBA |
| Methyl 10-trans,12-cis-octadecadienoate | (10E,12Z)-Methyl linoleate | 16.834 | 0.52 | $C_{19}H_{34}O_2$ | 294.5 | CPBA |
| 2-Oxabicyclo[3.2.0]hepta-3,6-diene | DTXSID30338464 | 3.836 | 0.49 | $C_8H_{10}O$ | 122.16 | CPBA |
| 7-Pentadecene | (E)-pentadec-7-ene | 21.479 | 0.36 | $C_{15}H_{30}$ | 210.3987 | CPBA |
| 1,2,3,4-Butanetetrol, [S-(R*,R*)]- | Erythritol, Lichen sugar | 3.955 | 0.31 | $C_4H_{10}O_4$ | 122.1198 | CPBA |
| N-Aminopyrrolidine | 1-Pyrrolidinamine | 6.484 | 0.23 | $C_4H_{10}N_2$ | 86.1356 | CPBA |
| Benzoic acid | Phenylcarboxylic acid | 6.331 | 0.21 | $C_7H_6O_2$ | 122.1213 | CPBA |
| γ-Sitosterol | Clionasterol | 27.123 | 12.41 | $C_{29}H_{50}O$ | 414.7067 | PGBE |
| Pyridine, 1,2,3,6-tetrahydro-1-(1-oxobutyl)- | 1-Butyryl-1,2,3,6-tetrahydropyridine # | 11.788 | 2.94 | $C_9H_{15}NO$ | 153.22 | PGBE |
| 9-Octadecenal, (Z)- | Olealdehyde | 21.543 | 2.25 | $C_{18}H_{34}O$ | 266.462 | PGBE |
| Vitamin E | 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-tridecyl)-,[2R-[2R*(4R*,8R*)]]- | 25.015 | 2.22 | $C_{29}H_{50}O_2$ | 430.7061 | PGBE |
| Cyclohexadecane | EINECS-206-041-2 | 16.805 | 1.73 | $C_{16}H_{32}$ | 224.4253 | PGBE |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | Pyranone | 5.788 | 1.43 | $C_6H_8O_4$ | 144.1253 | PGBE |
| Octadecanoic acid, 2,3-dihydroxypropyl ester | Glycerin 1-monostearate | 21.755 | 1.18 | $C_{21}H_{42}O_4$ | 358.5558 | PGBE |
| 3H-Pyrazol-3-one, 2,4-dihydro-5-methyl-2-phenyl- | Norphenazone | 4.964 | 1.15 | $C_{10}H_{10}N_2O$ | 174.1992 | PGBE |
| N-(2-Trifluoro-methylphenyl)pyridine-3-carboxamide oxime | N-hydroxy-N'-[2-(trifluoromethyl)phenyl]pyridine-3-carboximidamide | 24.752 | 1.1 | $C_{13}H_{10}F_3N_3O$ | 281.23 | PGBE |
| 2(1H)-Naphthalenone, octahydro-4 | 2-Decalone (cis-trans) | 21.493 | 0.68 | $C_{10}H_{16}O$ | 152.23 | PGBE |

However, on HPLC results presented in Table 4, it was found that FCBA observed to be positive for 11 compounds with maximum amount of Ferulic acid and Chlorogenic acid, LILE was positive for 11 compounds mainly Gallic acid, Chlorogenic acid and Caffeic acid, CPBA contain chiefly Caffeic

acid, Vanillic acid and Ferulic acid, while in PGBE 12 compounds observed and predominantly was Qurecitin, Gallic acid and Caffeic acid.

**Table 4: Quantification of Compounds by HPLC**

| Sr. No. | Name of Compound | FCBA | | LILE | | CPBA | | PGBE | |
|---|---|---|---|---|---|---|---|---|---|
| | | Retention Time | Conc. (PPm) | Retention Time | Conc. (PPm) | Retention Time | Conc. (PPm) | Retention Time | Conc. (PPm) |
| **1.** | **Qurecitin** | 3.02 | 4.57 | 3.02 | 73.55 | 2.907 | 18.16 | 3.12 | 180.07 |
| **2.** | **Gallic acid** | 4.773 | 1.43 | 4.527 | 128.11 | 4.547 | 61.25 | 4.787 | 94.41 |
| **3.** | **Caffeic acid** | 12.207 | 4.37 | 12.373 | 88.81 | 12.437 | 502.85 | 12.127 | 70.56 |
| **4.** | **Chlorogenic acid** | 15.333 | 60.57 | 15.88 | 100.4 | - | - | 15.98 | 72.15 |
| **5.** | **M.coumaric acid** | 19.973 | 3.21 | 19.827 | 14.8 | 20.36 | 15.75 | 19.753 | 17.89 |
| **6.** | **P. Coumeric acid** | 17.807 | 7.75 | 17.3 | 6.93 | - | - | 18.013 | 7.05 |
| **7.** | **Ferulic acid** | 22.38 | 80.21 | 22.153 | 44.67 | 22.12 | 167.67 | 22.487 | 60.01 |
| **8.** | **Cinamic acid** | 24.64 | 10.53 | 25.133 | 51.55 | 2.793 | 3.45 | 25.493 | 46.69 |
| **9.** | **Benzoic acid** | - | - | - | - | 4.673 | 2.34 | 16.06 | 30.65 |
| **10.** | **Syringic acid** | 16.54 | 16.9 | 16.76 | 13.66 | 16.633 | 43.51 | 26.12 | 8.64 |
| **11.** | **Sinapic acid** | 26.467 | 8.33 | 26.033 | 16.66 | - | - | 26.507 | 11.58 |
| **12.** | **Vanillic acid** | 13.447 | 3.68 | 13.04 | 12.4 | 13.637 | 237.52 | 13.52 | 56.67 |

‘-‘Absence of compound , FCBA-Ficus carica Bark Acetone, LILE- Lawsonia inermis Leaves Ethanol, CPBA-Carica Papaya Bark Acetone, PGBA- Pisidium guajava Bark Acetone

***Organoleptic evaluation of cream***

Total of eight parameters were followed to physically evaluate the cream. On visual observation, the cream was brown earthy in color with characteristic odor, uniform semisolid in homogeneity. The pH was found to be 6.4 which is good for skin, and was washable with tap water. On irritancy test, no redness, edema, itching and inflammation observed so found safe for skin use. The results for above evaluation attributes are shown in Table 5.

**Table 5: Organoleptic evaluation of cream**

| Sr. No. | Attributes | Observations | |
|---|---|---|---|
| 1. | Appearance | Brown earthy color |  |
| 2. | Odour | Characteristic | |
| 3. | Consistency | smooth | |
| 4. | Homogeneity | Uniform semisolid | |
| 5. | pH | 6.4 | |
| 6. | Spreadability | 7.3g cm/sec | |
| 7. | Washability | Easily removed with tap water | |
| 8. | Irritancy test | No redness, edema, inflammation | |

***4 weeks’ application of creams and measurement of results***

Cream D (with active ingredients) was applied on the affected area of D group and cream B (base cream) to the placebo group named as B group for 04 weeks. After the said time period the clinical parameters were measured as Objective parameter involves the extent of area as increase or decrease, intensity in terms of redness, swelling, crusting, scratch marks, skin thickening and dryness as mild, moderate and severe. The subjective parameter includes itch and sleeplessness as 0 to 10. The results measured and described in Table 6 with significant positive effects in D group while no change in group B except moderate dryness.

No serious adverse effects were observed during the study.

**Table 6: Results measured after application of creams for 4 weeks**

| Sr. No. | Clinical Parameter | Measurement | After D Cream application in D Group | After B Cream B Group |
|---|---|---|---|---|
| 1. | Extent area | Increase, Decrease | Decrease | No change |
| 2. | Redness | 0-3 (Mild, Moderate, Severe) | Mild | No change |

| | | | | |
|---|---|---|---|---|
| 3. | Swelling | 0-3 (Mild, Moderate, Severe) | Mild | No change |
| 4. | Crusting | 0-3 (Mild, Moderate, Severe) | Mild | No change |
| 5. | Scratch mark | 0-3 (Mild, Moderate, Severe) | Moderate | No change |
| 6. | Skin thickening | 0-3 (Mild, Moderate, Severe) | Mild | No change |
| 7. | Dryness | 0-3 (Mild, Moderate, Severe) | Mild | Moderate |
| 8. | Itch and sleeplessness | 0-10 (No to Worst) | No | No change |

Results showen in Table 7.

**Table 7: SEM of SCORAD values before application of cream and after the application of Cream in both D group and B Group**

| Sr. No. | Clinical Parameter | SEM of SCORAD Score Before Cream | SEM of SCORAD Score After Treatment D Group | SEM of SCORAD Score After Treatment B Group |
|---|---|---|---|---|
| 1. | Objective | 33.22±0.445 (±1.34%) | 16.612±0.268 (±1.72%) | 32.22±0.445 (±1.34%) |
| 2. | Subjective | 8.64±0.356(±4.12%) | 2.08±0.356(±4.12%) | 7.64±0.356 (±4.12%) |

With confidence interval of 95%, values measured for SCORAD Score after treatment of D group and B group

## Discussion

Skin bears the burden of several microorganisms and is an important part of immunity that protect against microorganism infections. Any damage or injury to the skin leads to easy penetration of bacteria and causes severe infections like folliculitis, dermatitis, etc. Usually, synthetic antimicrobial ointments, which are expensive and have several side effects are used. Recently, the number of skin infections are increasing due to various factors and the trend of polyherbal formulations having antimicrobial properties is also growing worldwide to counter these infections as an alternative of synthetic ointments (Joshi et al., 2021).

The present work was the formulation of polyherbal cream, its organoleptic evaluation and then single blind study. Research was focused on investigating the treatment of dermatitis patients by decreasing the SCORAD score. The cream formulated was oil in water emulsion. The results measured from above research indicated the beneficial effects of cream D in dermatitis patients. The productive results in treatment of dermatitis by cream D is due to the presence of compounds observed on HPLC, as these have significant biological activities as antimicrobial, antioxidant, anti-inflammatory, anti-dermatitis, anti-cancer and wound healing (Magnani *et al*., 2014). Vanillic acid, ferulic acid, Qurecitin, Cholorogenic acid areused in skin medications due to analgesic, anti-aging, skin wound healing effects and also inhibit multidrug efflux system in resistant bacteria and prevents from UV radiation (Zduńska *et al*., 2022). A previous study was also found in which a polyherbal formulation was used against the skin infection psoriasis and found remarkable results. Polyherbal cream is not harmful or irritant and is easily removeable after application to the skin Herbal medicines are used from the time of origin and trend of herbal formulation like cream is also increasing worldwide. Poly herbal formulation results are more promising as compared to individual herbs due to synergize effect (Sonalkar et al., 2016). The present study revealed remarkable results against dermatitis and successfully reduced the skin swelling and redness after 4 weeks of application. Similar results were also found in the previous study against diabetic wounds using herbal ointment formulations (Nehete et al., 2016).

Therefore, based on the findings, it can be inferred that the components used in the poly herbal cream formulation have the potential to effectively treat skin infections such as dermatitis. In the future, with further modifications and advancements, this could represent a significant milestone in the field of medicine.

**Conclusion:**
For the treatment of skin diseases, the above mentioned plants can be used in the form of crude drug or by isolating compounds for preparing medicines. The polyherbal cream formulated and evaluated in this research will be a revolution in skin medicines and mainly is cost effective. The results on patients of dermatitis was remarkable mainly by decreasing the extent area, itching and inflammation.

**Acknowledgement:**
I would like to express my deepest gratitude to the subjects involved as their support and nurturing has made this project a success.

**Funding:**
The author(s) received no financial support for the research, authorship, and/or publication of this article.

**Conflict of interest:**
The author declares that there is no conflict of interest.

**Refrences**
1. Thomsen SF. Atopic dermatitis: Natural history, diagnosis, and treatment. *ISRN Allergy*. 2014; 2014:1-7. doi:10.1155/2014/354250
2. Sroka-Tomaszewska J, Trzeciak M. Molecular mechanisms of atopic dermatitis pathogenesis. *Int J Mol Sci*. 2021;22(8):4130. doi:10.3390/ijms22084130
3. Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet*. 2006;368(9537):733-743. doi:10.1016/S 0140-6736(06)69283-0
4. Frazier W, Bhardwaj N. Atopic Dermatitis: Diagnosis and Treatment. *Am Fam Physician*. 2020;101(10):590-598. Accessed October 29, 2023.
5. Segre J, Kong H, Tirosh O, Byrd A. Human Skin Microbiome: Integrating bacterial, fungal and viral communities. *FASEB J*. 2019;33(S1). doi: 10.1096/fasebj.2019.33.1_supplement.93.4
6. Dréno B, Araviiskaia E, Berardesca E, et al. Microbiome in healthy skin, update for dermatologists. *J Eur Acad Dermatol Venereol*. 2016;30(12):2038-2047. doi:10.1111/jdv.13965
7. Paller AS, Kong HH, Seed P, et al. The microbiome in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2019;143(1):26-35. doi: 10.1016/j.jaci.2018.11.015
8. Nakatsuji T, Gallo RL. The role of the skin microbiome in atopic dermatitis. *Ann Allergy Asthma Immunol*. 2019;122(3):263-269. doi: 10.1016/j.anai.2018.12.003
9. Tabassum N, Hamdani M. Plants used to treat skin diseases. *Pharmacogn Rev*. 2014;8(15):52. doi:10.4103/0973-7847.125531
10. Avena-Woods C. Overview of atopic dermatitis. *Am J Manag Care*. 2017;23(8 Suppl): S115-S123.
11. Silverberg JI, Gelfand JM, Margolis DJ, et al. Patient burden and quality of life in atopic dermatitis in US adults: A population-based cross-sectional study. *Ann Allergy Asthma Immunol*. 2018;121(3):340-347. doi: 10.1016/j.anai.2018.07.006
12. Xu X, van Galen LS, Koh MJA, et al. Factors influencing quality of life in children with atopic dermatitis and their caregivers: a cross-sectional study. *Sci Rep*. 2019;9(1):15990. doi:10.1038/s41598-019-51129-5
13. Bieber T. Atopic dermatitis: an expanding therapeutic pipeline for a complex disease. *Nat Rev Drug Discov*. 2022;21(1):21-40. doi:10.1038/s41573-021-00266-6
14. Tsioutsiou EE, Amountzias V, Vontzalidou A, et al. Medicinal plants used traditionally for skin related problems in the South Balkan and East Mediterranean region—A review. *Front Pharmacol*. 2022;13. doi:10.3389/fphar.2022.936047

15. Awad El-Gied A, Abdelkareem A, Hamedelniel E. Investigation of cream and ointment on antimicrobial activity of Mangifera indica extract. *J Adv Pharm Technol Res*. 2015;6(2):53. doi:10.4103/2231-4040.154530
16. Badoni Semwal R, Semwal DK, Combrinck S, Cartwright-Jones C, Viljoen A. Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects. *J Ethnopharmacol*. 2014;155(1):80-103. doi: 10.1016/j.jep.2014.05.042
17. Niazi M, Mehrabani M, Namazi MR, et al. Efficacy of a topical formulation of henna (Lawsonia inermis L.) in contact dermatitis in patients using prosthesis: A double-blind randomized placebo-controlled clinical trial. *Complement Ther Med*. 2020;49(102316):102316. doi: 10.1016/j.ctim.2020.102316
18. Mir SR, Mohammed Ali MZ, Ahmad S. *NEW DIGLUCOSIDE AND PHENOLIC GLYCOSIDIC ESTERS FROM THE STEM BARK OF FICUS CARICA L*.; 2023.
19. Fatima U, Shahid S. Pharmacological activities of Carica papaya Linn. *J Basic Appl Sci*. 2018; 14:210-216. doi:10.6000/1927-5129.2018.14.33
20. Vij T, Prashar Y. A review on medicinal properties of Carica papaya Linn. *Asian Pac J Trop Dis*. 2015;5(1):1-6. doi:10.1016/s2222-1808(14)60617-4
21. Joseph B, Priya RM. Phytochemical and Biopharmaceutical Aspects of Psidium guajava (L.) Essential Oil: A Review. *Res J Med Plant*. 2011;5(4):432-442. doi:10.3923/rjmp.2011.432.442
22. Growther L, K. S. Phytochemical analysis and antimicrobial properties of Psidium guajava leaves and bark extracts. *Asian J Pharm Pharmacol*. 2018;4(3):318-323. doi:10.31024/ajpp.2018.4.3.13
23. Matangi S, Santhosh Aruna Mamidi STV, Raghavamma RR. Formulation and evaluation of anti-aging poly herbal cream. *skin*. 2014;5(6).
24. Chavan P, Kalshetti M, Navindgikar N. Formulation and evaluation of polyherbal cream. *International Journal of Current Pharmaceutical Research*. Published online 2020.
25. Akhtar eN. Evaluation of various functional skin parameters using a topical cream of Calendula officinalis extract. *Afr J Pharm Pharmacol*. 2011;5(2):199-206. doi:10.5897/ajmr10.368
26. Joshi, P., Joshi, S., Rajani, U., Semwal, R. B., & Semwal, D. K. (2021). Formulation and evaluation of polyherbal cream and lotion for the treatment of psoriasis-induced secondary infections. *Current Reviews in Clinical and Experimental Pharmacology Formerly Current Clinical Pharmacology*, *16*(1), 79-96.
27. Magnani C, Isaac VLB, Correa MA, Salgado HRN. Caffeic acid: a review of its potential use in medications and cosmetics. *Anal Methods*. 2014;6(10):3203-3210. doi:10.1039/c3ay41807c
28. Zduńska-Pęciak K, Dębowska R, Kołodziejczak A, Rotsztejn H. Ferulic acid-A novel topical agent in reducing signs of photoaging. Dermatologic Therapy. 2022;35(7).
29. Sonalkar, M. Y., & Nitave, S. A. (2016). Formulation and evaluation of polyherbal cosmetic cream. *World J Pharm Pharm Sci*, *5*, 772-9.
30. Nehete, M. N., Nipanikar, S., Kanjilal, A. S., Kanjilal, S., & Tatke, P. A. (2016). Comparative efficacy of two polyherbal creams with framycetin sulfate on diabetic wound model in rats. *Journal of Ayurveda and Integrative Medicine*, *7*(2), 83-87