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# ANTI-PSORIATIC ACTIVITY AND HPTLC FINGERPRINTING OF PREPARED POLY-HERBAL CREAM

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**Keywords:**

Psoriasis, β-sitosterol, Psoralen, polyherbal extract

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**ABSTRACT:** The current investigation's goal is to prepare polyherbal cream by using plant extracts of *Terminalia chebula, Sapindus trifoliatus, Psoralea corylifolia* and carry out high-performance thin layer chromatography fingerprinting to confirm that the corresponding extracts utilized as standards contain the active ingredients. Cream of plant extract prepared by using fusion method and the prepared cream formulation was examined qualitatively in order to determine whether any active phytoconstituents were present and its anti-psoriatic activity. Thus, high-performance thin layer chromatography fingerprinting emerged as crucial instrument for identification, subjective analysis, a phytochemical evaluation of polyherbal cream of *Terminalia chebula, Sapindus trifoliatus, Psoralea corylifolia* seed extracts. Prepared polyherbal cream characterised by different parameters including irritancy test, wash ability test, phase separation and spreadability. The present study concluded that the High-performance thin layer chromatography studies showed presence of β-sitosterol and psoralen in prepared polyherbal cream. Prepared herbal cream showed the most promising anti-psoriatic effect. Active constituents responsible for treating psoriasis were present in the obtained polyherbal extract.

**INTRODUCTION:** The Indian traditional health care system includes a variety of herbal remedies made from plants with medicinal properties that have been used for ages. They are suggested because of their engaging multidimensional activities.

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Herbal medications have relatively minor adverse effects as compared to synthetic drugs. The herbal remedy is widely accessible and simple to apply. Herbal remedies are essential for treating skin and inflammatory conditions.

A change in food and lifestyle may help alleviate the symptoms of psoriasis, according to certain research. Plants such as *Echinacea angustifolia, Echinacea purpurea, Sapindus trifoliatus, Lavendula officinalis, Azadirachta indica, Achyranthes aspera, Sarco asoca, Terminalia chebula, Matricaria chamomile* and *Psoralea*

*corylifolia, Solanum nigrum, Annona squamosa, Aloe vera* are used in the treatment of psoriasis[1]. Allopathic medications such as corticosteroids, methotrexate, and immune suppressants reduce inflammation, cell proliferation, scales, and plaque in psoriasis; however, side effects are more severe than drug efficacy. Long-term drug use can result in major side effects like hepatotoxicity and renal failure, which can occasionally be fatal. Therefore, herbal medications are safe to use in the treatment of psoriasis because they have negligible or no side effects and a therapy cost that is comparable to that of allopathic medicine. Some of medicinal plants used in Ayurvedic preparations have undergone extensive research to determine their therapeutic action, while others require more investigation.

Only the physicochemical parameters are primarily specified in plant material monographs found in various pharmacopeias. Therefore, appropriately standardizing herbal extracts and their preparation may benefit from application of contemporary techniques in order to recognize and measure the active ingredients in plant material [2]. The need to apply appropriate standards and contemporary controlled methods to guarantee quality of medicinal herb products has also been underlined by the World Health Organization. A fingerprint generated by high-performance thin- layer chromatography has superior resolution and can estimate active constituents with reasonable accuracy in a shorter amount of time. To address the need for a more potent and effective quality assessment, the chromatographic fingerprint is a sensible choice. Accordingly, three plants were selected for the studies including the seeds of *Terminalia chebula*, seeds of *Psoralea corylifolia*, and seeds of *Sapindus trifoliatus*. The plants are reported to have very good activity in treating various skin diseases [3].

A moderate tree in the Compositaceae family is *Terminalia chebula*. It is frequently referred to as an ink tree or black myrobalan. In India and other Asian and African nations, traditional medicine makes extensive use of it [4]. The main justification for its use is its extensive pharmacological effect, which is connected to the physiologically active chemicals present in the plant [5]. The triterpenes arjun glucoside 1, chebulosides 1 and 2, arjungenin are found in *Terminalia chebula*. Tannic acid (20–40%), chebulinic acid (30%), luteolin, rutins, and quercetin, flavonoids, ellagic acid, gallic acid, ethyl gallate, and terchebin are other components. There is also some purgative of the anthraquinone type [6]. Numerous illnesses, including leprosy, cancer, paralysis, cardiovascular disorders, and ulcers are treated with it. It was proven to have anti-oxidant activity [7]. One of the most widely used traditional Chinese medicines that are formally recognized by the Chinese Pharmacopoeia is *Psoralea corylifolia* Linn. *P. corylifolia* is an annual herb found in the Indian plains. Many skin conditions, including psoriasis, leukoderma, and leprosy, have been treated with it for many years [8]. Limonene, α-element, γ-element, β-caryophylenoxide, 4- terpineol, linalool, geranyl acetate, angelicin, bakuchiol, psoralen, and iso psoralen are among the constituents of the essential oil. For ages, the *Psoralea corylifolia* plant has been used to treat vitiligo and leukemia [9]. It's said to be an essential oil. When exposed to sunlight, the psoralens- containing furanocoumarins increase the skin's production of melanin pigment [10].

*Sapindus trifoliatus* Linn is one of the oldest cultivated plants in the world. It belongs to the family Sapindaceae. They are commonly known as soap berries or soap nuts because the pulp of the fruit is used to make soap. The methanolic and aqueous seed extracts of the plant *Sapindus trifoliatus* was found to contain triterpenoids, flavonoids, carbohydrates, and steroids. Seeds are found to contain fatty acids. Glucopyranosides of Stigmasterol, Kaempferol, quercetin, ß- sitosterol, Hederagenin, protein, carbohydrate, and Starch are also found to be present in the plant. Scientific research has demonstrated the anti-ulcer, anti-parasitic, anti-inflammatory, muscle-relaxant, anti-diabetic, anti-fungal, anti-acne, and anti-dandruff properties of *Sapindus trifoliatus*. The proposed work aimed to investigate the extracts' HPTLC fingerprinting and the preparation of corresponding topical polyherbal creams [11].

## MATERIALS AND METHODS:

### Materials:

**Collection and Authentication of Plant Material:** The plant materials *Terminalia chebula* (Seeds), *Sapindus trifoliatus* (Seeds), *Psoralea corylifolia* (Seeds) were gathered from nearby regions of Hyderabad and verified by Osmania University's

Department of Botany. The authentification ID for the plant is 0215/0266/0167.

**Methods:**

**Extraction of Plant Material:** All three plant materials collected were shade-dried and powdered in a pulverizer and subjected to successive solvent extraction using solvents ethanol, n-hexane, chloroform, and ethyl acetate by soxhlet extraction. A rota evaporator was used to concentrate the extracted materials. The concentrated extracts were stored in a refrigerator. The color and percentage yield of each extract were examined [12].

**Preliminary Phytochemical Screening of Extracts:** These extracts were further subjected to phytochemical investigation of metabolites like carbohydrates, alkaloids, glycosides, flavonoids, tannins, proteins, steroids, *etc*. which are responsible for therapeutic effects [13].

**Preparation of Poly Herbal Cream Formulation:** Herbal extracts of N-hexane and Ethyl acetate from all three plants were used to prepare cream. The fusion method was used to prepare the creams. Using this method, the base constituents were combined and melted over a water bath that was heated to 70°C. Following the melting process, the ingredients were stirred gently while keeping the temperature at 70°C for approximately five minutes. After the mixture cooled to 45°C, the necessary number of extracts was added and thoroughly mixed. The prepared creams were used for additional research after being placed in aluminium tubes, labelled, and kept at room temperature. For the ethyl acetate and N-hexane extract preparations, the labels were NF1 and NF2, respectively [14]. **Table 1** displays the formulation table and **Fig. 1** displays the photographs of the prepared herbal cream.

**TABLE 1: TABLE OF INGREDIENTS FOR HERBAL CREAM**

| Sr. no. | Ingredients | Quantity (1% w/w) | Quantity (3% w/w) | Role of Ingredients |
|---|---|---|---|---|
| 1. | Herbalextract | 1gm | 3gm | Crude drug |
| 2. | White Wax | 50gm | 48gm | Emulsifier |
| 3. | Liquid Paraffin | 0.7gm | 0.7gm | Emollient |
| 4. | Borax | 20gm | 20gm | Stabilizer |
| 5. | Purifiedwater | 28.3ml | 28.3ml | Vehicle |

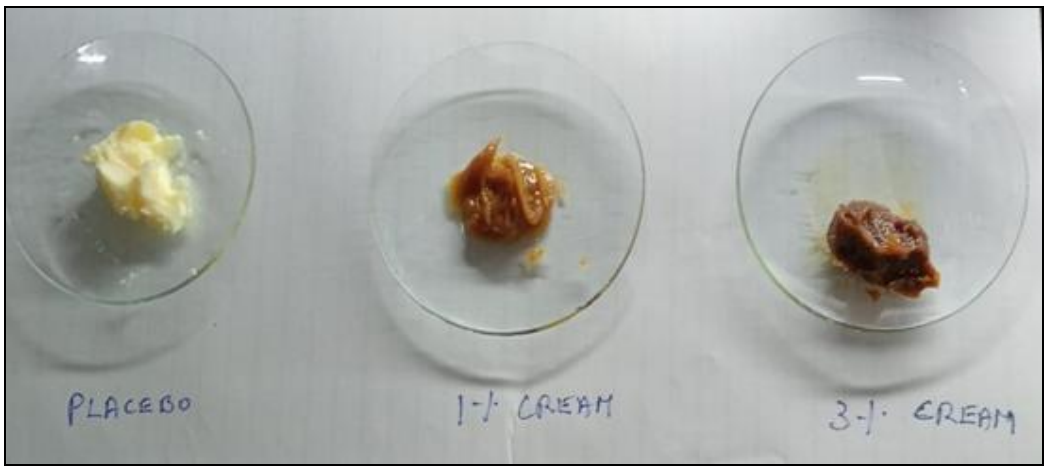



**FIG. 1: FORMULATION IMAGES OF HERBAL CREAM**

**Evaluation of Prepared Herbal Cream:**

**Physical Parameters:** Prepared herbal cream tested for physical parameters including color, odour, texture, consistency, form of physical state and pH were observed [15].

**Irritancy:** On the dorsal surface of the left hand, mark the area (1 cm2). The area was then covered with the cream, and the time was recorded. The irritating impact is tested after a 24-hour interval [15].

**Washability:** A little amount of cream was applied to the hand, and it was then washed with tap water to test the hand's washability [15].

**Phase Separation:** The prepared cream is maintained in a tightly sealed container at room temperature, shielded from the sun, and left to be observed for phase separation for 24 hours [15].

**Spreadability:** Spreadability testing is done on the NF1 and NF2 formulations. The better the spreadability, the faster the two slides can be separated [15].

**Anti-psoriatic Activity of Prepared Herbal Cream Psoriasis Induction:** Shaving was used to get rid of hair on the dorsal skin. UV light (385 nm) was applied to 10% of the body's surface area for 30 minutes at a vertical distance of 20 cm. Following irradiation, rats were observed to note any alterations in the skin, the emergence of skin lesions, and any other distinct behaviors. In all, 24 Wistar rats were employed in the investigation. As previously indicated, psoriasis was induced in animals. Following induction, the animals were split up into five groups, each with six animals (n = 6) in it [16, 17] Group 1: Normal control (vehicle-treated, non-induced psoriasis)

Group 2: Disease control (no therapy; psoriasis-induced).

Group 3: The tretinoin group (0.05% tretinoin gel o.d. for 12 days; psoriasis caused).

Group 4: 1% Polyherbal cream (used o.d. for 12 days to treat psoriasis-induced inflammation).

Group 5: 3% poly herbal cream (Psoriasis induced, treated with 3 % polyherbal creamod for 12 days).

Albino Rats were employed in the screening process. Poly herbal creams containing 1% and 3% extracts of ethyl acetate and N-hexane were screened in relation to the standard (Tretinoin gel 0.05%). For twelve days, polyherbal creams were administered once a day, five times a week. For twelve days, the standard medication was given topically once a day, five times a week. Every fourth day, the animals were assessed based on the psoriatic lesion severity index.

A severity-based visual scoring system was created. A scale of 0 to 3 was used to assess SI, with 1 denoting mild redness, 2 denoting moderate redness and erythema, and 3 denoting severe redness, erythema, and scaling [18]. After the tissues were removed, they were preserved in formalin at -20°C for the biochemical assessment of the hydroxyproline level. Following a three-hour treatment with 6N HCl at 1300 c, tissue samples were hydrolysed. After the hydrolysate was neutralized to PH 7.0, it spent 20 minutes oxidizing with chloramine-T. Each test tube was filled with 2.5 mL of Ehrlich reagent five minutes after the oxidizer was applied. The test tubes were then submerged in a water bath and allowed to cool before receiving 6.6 mL of isopropyl alcohol each. Following a thorough agitation, the samples were examined at a wavelength of 557 nm in 1 cm corvettes using a spectrophotometer. The samples were compared to a control group in which water was used in place of the solution under analysis. For four hours, the color that has evolved essentially does not alter significantly. Each sample that was analysed had its hydroxyproline content determined using a calibration curve [19].

**Thickening of the Epidermis and Histopathology:** Animals were put to sleep with ketamine at the conclusion of the investigation. Skin (tissue) specimens were gathered and kept in glass vials with a 10% formalin solution. Skin biopsies from the lesion regions were examined under a light microscope to measure the thickness of the stratum corneum and the cellular portion of the epidermis. Haematoxylin-eosin dye was used to stain longitudinal sections of skin specimens (about 5 mm thick) for histopathology. Using a calibrated ocular micrometre, the thickness of the cellular portion of the epidermis was measured, and all data were corrected for magnification optics [20].

**HPTLC Fingerprinting of Prepared Polyherbal Cream Formulation:** To confirm that the polyherbal cream contains active components, HPTLC studies were conducted using the corresponding extracts as the standard. As an official technique for evaluating drug content homogeneity, assay value, purity, dissolution, drug-drug interaction, and bioavailability, HPTLC is recognized by most pharmacopeias.

These days, practically all research labs have an HPTLC system. It manages multiple samples concurrently, even those with different compositions and characteristics, enabling multiple analyses to be performed at once. It can be considered to a time machine that accelerates tasks that are typically too difficult to complete with other analytical methods.

**Chromatographic Conditions for the Identification and Quantification of β-sitosterol and Psoralin in the Polyherbal Creams:** Plate layout: For β-sitosterol and Psoralin Stationary phase: Merck, HPLC silica gel 60 F 254 Plate format: 200 X 100 mm

Application type: User

Application: Position Y: 8.00 mm, Length: 8.00 mm

Track: 18.4 mm between first position and distance X 23.00 mm Solvent front position: 70 mm

Application of Sample: Using the automated sample application device Camag Linomat IV, the sample was applied as a band with a 9 mm bandwidth and 9 mm spacing. Preparation of standard solutions: 1 mg/ml in methanol.

**Preparation of Samples:** 1000mg/10 ml in methanol developing distance: 70mm from the lower edge of the plate.

**Derivatizing Reagent:** Anisaldehyde Sulphuric Acid Reagent for β-Sitosterol.

**Selection of Mobile Phase:** Toluene: Ethyl acetate (08:02) for Psoralen and Chloroform: Ethylacetate: Methanol: Water (3:8:4.4:1.8) for β-Sitosterol.

**HPTLC Development:** A densitometric assessment system with CAT software, the CAMAG TLC Scanner IV, was used to scan items in the reflectance or transmission mode of thin-layer chromatograms by absorbance or by fluorescence at 254 nm. The fingerprint of the HPTLC profile was taken using the computer printer [21, 22, 23].

## RESULTS AND DISCUSSION:

**Preliminary Phytochemical Evaluation:** Chloroform, ethyl acetate, N-hexane, and ethanol are used for the initial extraction of each plant material. An extract from Soxhlet is subsequently concentrated. The results of the calculations to determine the percent yield from the dried extracts are provided in **Table 1**.

Extraction of plant material by using N-hexane and ethyl acetate solvent showed a high percent yield as compared to the extraction by using the solvent chloroform and ethanol. A preliminary phytochemical investigation is carried out on the obtained extract to ascertain the presence or absence of various phytoconstituents, including lipids, proteins, carbohydrates, amino acids, sterols, triterpenes, cardiac glycosides, and flavonoids. **Table 2** and **3** contain tabulations of these investigations.

**TABLE 2: THE PERCENTAGE YIELD FOLLOWING EXTRACTION**

| Sr. No. | Drug | Solvent | Color | % Yield |
|---|---|---|---|---|
| 1. | | N-hexane | Yellow | 13.80 |
| 2. | | Chloroform | Yellow | 05.55 |
| 3. | *Sapindus trifoliatus* | Ethyl acetate | yellow | 12.25 |
| 4. | | Ethanol | brown | 10.00 |
| 5. | | N-hexane | brown | 08.20 |
| 6. | | Chloroform | Yellow | 07.50 |
| 7. | *Terminalia chebula* | Ethyl acetate | Green | 11.80 |
| 8. | | Ethanol | Brown | 04.90 |
| 9. | | N-hexane | brown | 10.70 |
| 10. | | Chloroform | Yellow | 09.10 |
| 11. | *Psoralea corylifolia* | Ethyl acetate | Brown | 11.50 |
| 12. | | Ethanol | Pink | 06.50 |

**TABLE 3: PHYTOCHEMICAL EVALUATION**

| Name of thetest | N-Hexane | Chloroform | Ethyl acetate | Ethanol |
|---|---|---|---|---|
| Carbohydrates | + | + | - | + |
| Alkaloids | + | - | + | + |
| Glycosides | - | - | + | + |
| Flavonoids | - | - | - | + |
| Tannins | + | - | - | + |
| Proteins | + | - | - | - |
| Steroids and triterpenoids | - | - | + | + |
| Phenolic compounds | + | + | - | + |

Based on physicochemical and pharmacological evaluation, two formulations were selected for further study. Two varieties of poly herbal creams are available: NF1, which contains 1% polyhexane extract and ethyl acetate extract from all three plants, and NF2, which contains 3% polyhexane extract and ethyl acetate extract from all three plants.

**Evaluation Parameters:** Results of Evaluation parameters are reported in **Table 4.**

**TABLE 4: EVALUATION PARAMETERS**

| Evaluation Parameters | NF 1 | NF 2 |
|---|---|---|
| Colour | Yellow | brown |
| Odour | Pleasant | Pleasant |
| Texture | smooth | smooth |
| consistency | semisolid | semisolid |
| pH | 6.12 | 6.22 |
| Irritant effect | Nil | Nil |
| Wash ability | Easily washable | Easily washable |
| Separation of phase | Absence of phase separation | Absence of phase separation |
| Spreadability (gcm/sec) | 2.5 | 3 |

**Anti-psoriatic Activity of Prepared Herbal Cream:** Plant extracts in n-hexane and ethyl acetate were combined to create a cream with 1% and 3% weight/weight concentrations, respectively. This mixture was dubbed NF1 & NF2 extract. The creams were then pharmacologically evaluated for their anti-psoriatic activity using Perry's mouse tail method. The cream showed anti-psoriatic activity which was comparable to standard Retino ointment. The current investigation verifies the anti-psoriatic properties of extracts from the seeds of *Terminalia chebula, Sapindus trifoliatus,* and *Psoralea corylifolia* using n-hexane and ethyl acetate. **Fig. 2** shown the Histopathology study of conducted work of psoriasis activity.

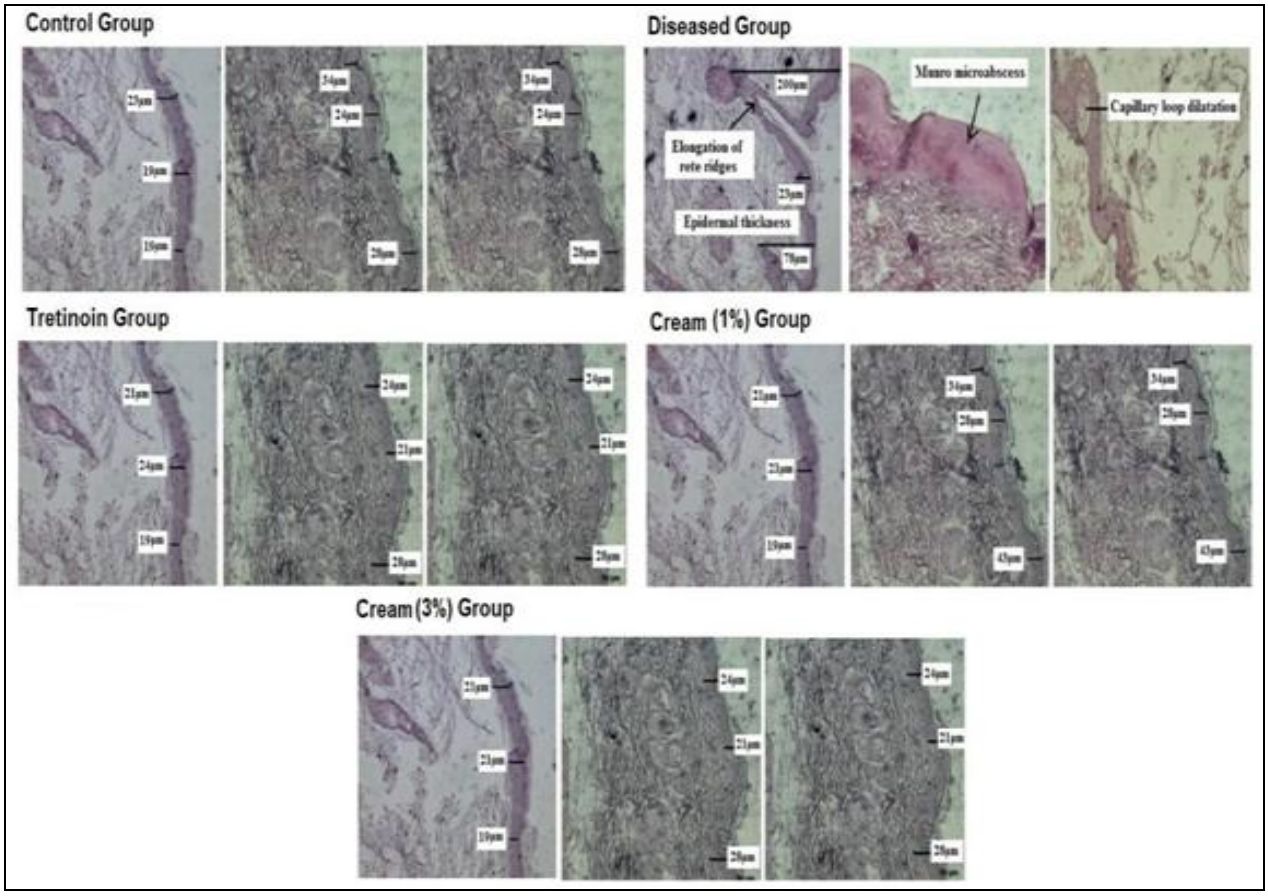



**FIG. 2: HISTOPATHOLOGY STUDY**

**TABLE 5: IMPACT OF TOPICAL FORMULATIONS ON THE SEVERITY INDEX OF RAT PSORIASIS CAUSED BY UV RADIATION**

| | Severity Index | | | | | |
|---|---|---|---|---|---|---|
| **Groups** | **Day 4** | **% Decrease** | **Day8** | **% Decrease** | **Day12** | **% Decrease** |
| Normal | 0.0±0.0 | _ | 0.0±0.0 | _ | 0.0±0.0 | _ |
| Diseased | 3.2±0.52 | _ | 1.8±0.41 | _ | 0.67±0.52 | _ |
| Tretinoin | 2.5±00 | 21.8 | 1.2±0.41 | 33.3 | 0.0±0.0 | 100 |
| Sample –1% cream | 2.8±0.61 | 12.5 | 1.51±0.51 | 16.11 | 0.42±0.51 | 37.3 |
| Sample –3% cream | 2.6 ±0.56 | 18.7 | 1.32±0.51 | 26.6 | 0.11±0.51 | 83.5 |

The findings clearly demonstrated that the severity index decreased day over day for the 1%, 3% screams and tretinoin group. Day 4 results showed that the group receiving tretinoin treatment had 21.8% psoriatic lesions, compared to 12.5% and 18.7% for the groups receiving 1% and 3% cream. Day 8 results showed that the percentage drop in the severity index for the Tretinoin was 33.3%, and for the 1% and 3% cream, it was 16.1% and 26.6%, respectively. On day twelve, the tretinoin group treated every psoriatic lesion (100%) while the 1% and 3% treated creams 37.3% and 83.5% of psoriatic lesions, respectively.

**TABLE 6: SUMMARY OF HISTOPATHOLOGY**

| **Groups** | **MunroMicro abscess** | **Elongation of Rete ridges** | **Capillaryloop dilatation** | **Epidermal thickness (µm)** |
|---|---|---|---|---|
| Normal | _ | _ | _ | 24.3 |
| Diseased | + | +++ | +++ | 78.0 |
| Tretinoin | _ | _ | _ | 33.9 |
| Sample – 1%cream | - | - | - | 38.9 |
| Sample – 3%cream | - | - | - | 36.8 |

+ Mild lesion, ++ Moderate lesion, +++ Severe lesion, - No lesion

**Hydroxyproline Content:** Hydroxyproline content: The metabolism of collagen proteins is drastically altered in psoriatic lesions as a result of inflammation-induced turnover acceleration. A small number of studies have reported elevated prolidase activity in psoriatic lesions as a result of collagen degradation. Additionally, prolidase activity lowers the amount of hydroxyproline in psoriatic lesions, and hydroxyproline is a psoriasis biomarker. It is clear from the data that the sick group's hydroxyproline content levels (12.9±0.21 microgram/100 g tissue) are significantly lower than those of the normal control group (19.8±0.32 microgram/100 a g tissue). The biochemical analysis showed that, although the hydroxyproline content is lower than in the normal control group (19.8±0.34 microgram/ 100 g tissue), it is similar in the tretinoin group (16.8±0.26 microgram/100 g tissue), 1% cream (15.11±.34 microgram/100 g tissue), and 3% cream (16.11±0.56 microgram/100 g tissue). This suggests that 1% and 3% cream can restore collagen and have anti-inflammatory properties. **Fig. 3** displays the hydrohyproline content of the various groups.

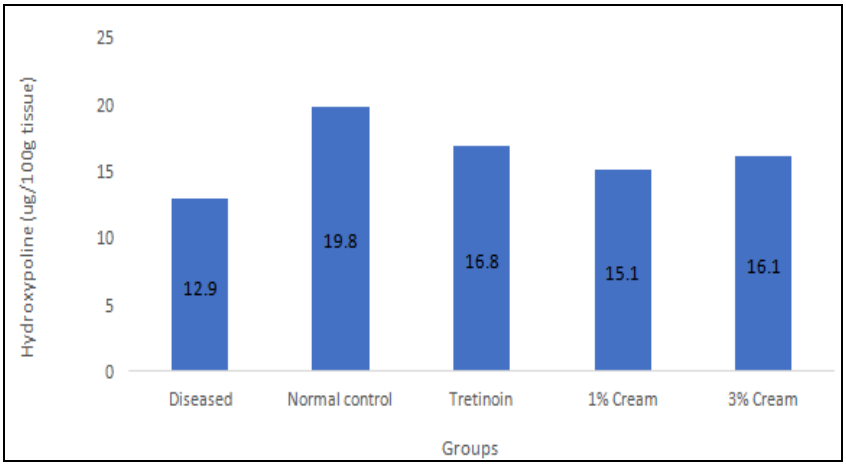
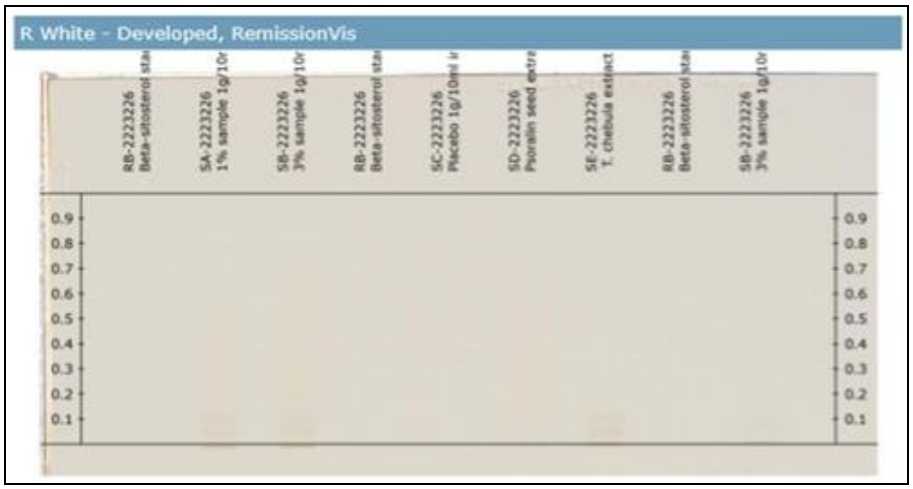
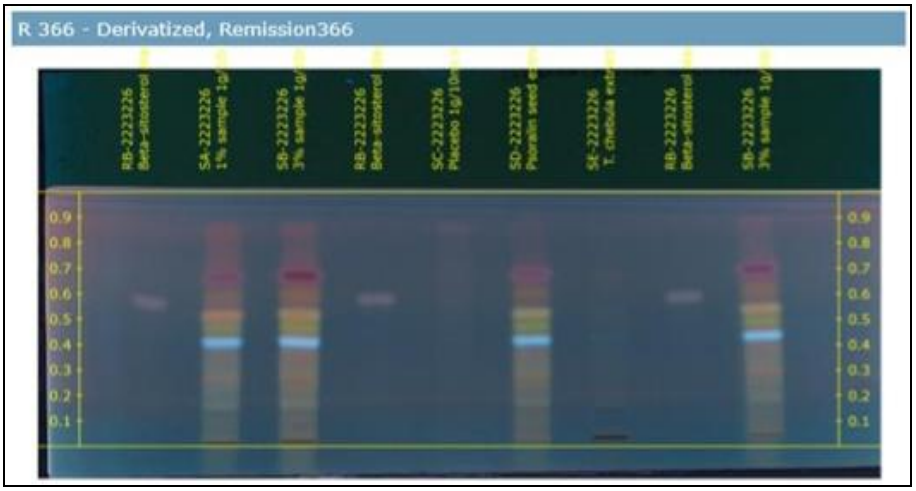



**FIG. 3: CHART OF HYDROHYPROLINE CONTENT OF DIFFERENT GROUPS**

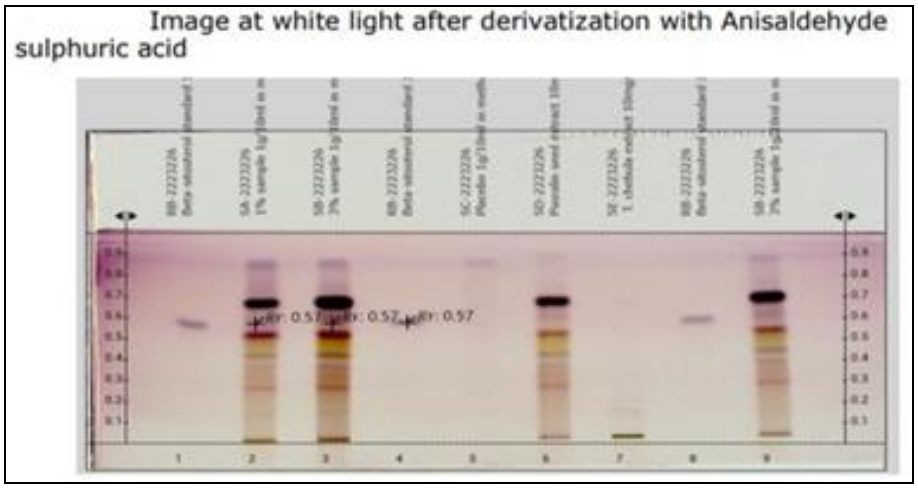
**HPTLC Fingerprinting for Prepared Polyherbal Cream:** Standardization of formulation of NF1& NF2 was carried out based on HPTLC fingerprints. N-hexane and ethyl acetate extract formulations were subjected to HPTLC fingerprinting utilizing the WINCHROME CAMAG equipment, LINOMAT V sample applicator, and WIN CATS software. **Fig. 4** and **5** display the chromatograms that are the outcome. At the Rf value, overlapping peaks were discovered, and the remaining portion of the scan displayed distinctive, recognizable peaks. Similar scans have been seen in formulations.




**(A)**

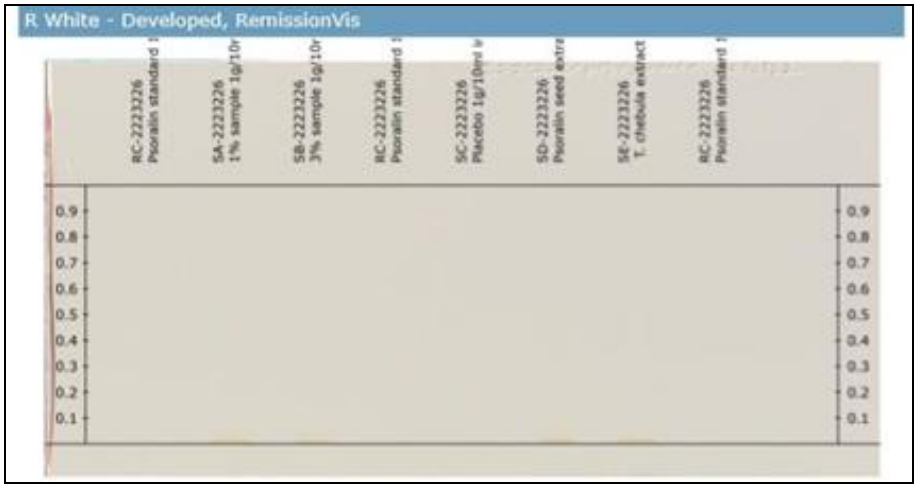



**(B)**

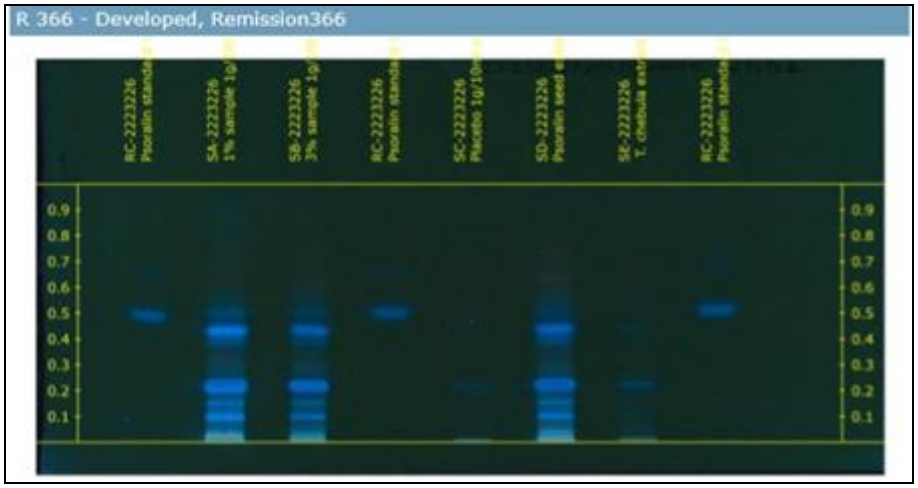



**(C)**

**FIG. 4: HPTLC SCAN FINGERPRINT OF B-SITOSTEROL, 1% & 3% POLYHERBAL CREAM (A, B, C)**




**(A)**

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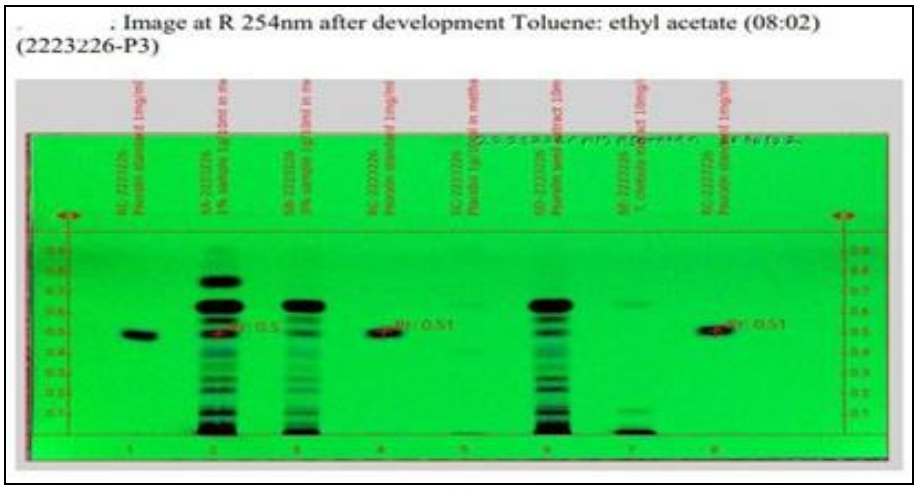


**(C)**

**FIG. 5: HPTLC SCAN FINGERPRINT OF PSORALIN, 1% & 3% POLYHERBAL CREAM (A, B, C)**

Single-level quantification was done for the detected β-sitosterol and Psoralen. After the development of plate with Chloroform: ethyl acetate: methanol: water (3:8:4.4:1.8) and derivatize with Anisaldehyde sulphuric acid reagent β-sitosterol was detected in the given a 1% cream and a 3% cream sample at Rf value 0.57. 0.010% and 0.015% of β-sitosterol were quantified in 1% cream sample and 3% cream sample respectively. After the development of the plate with Toluene: ethyl acetate (08:02) psoralen was detected in the given 1% and 3% of the cream sample at Rf value 0.51. 0.023% and 0.060% of psoralen were quantified in a 1% cream sample and

a 3% cream sample respectively. The manufactured cream formulations of NF1 and NF2 are stable and efficacious for the duration of their shelf life, according to this study's findings.

**CONCLUSION:** Extraction of plant material by using N-hexane and ethyl acetate solvent showed a high percent yield as compared to the extraction by using the solvent chloroform and ethanol. It was discovered that the main chemical components of these plants in N-hexane and ethyl acetate extracts were flavonoids and steroids. The phytochemical analysis using high- performance thin-layer chromatography fingerprinting helps standardize polyherbal cream preparation containing seed extracts of *Sapindus trifoliatus*, *Terminalia chebula*, and *Psoralea corylifolia*. To determine if β-sitosterol and psoralen were present in the NF1 and NF2 formulations with their corresponding extracts, high-performance thin layer chromatography fingerprinting was used. Psoralen and β-sitosterol were identified via early phytochemical screening in the N-Hexane and ethyl acetate extracts. Skin thickness and scaling were significantly reduced in the treated groups after treatment with n-hexane and ethyl acetate extracts of *Terminalia chebula, Sapindus trifoliatus* and *Psoralea corylifolia*. This indicated a significant improvement in psoriasis. The therapeutic efficacy of cream formulations containing polyherbal extract is confirmed by the presence of phytoconstituents such as psoralen and β-sitosterol.

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**CONFLICT OF INTERESTS:** Declared none

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