Fitoterapia

LINKING PHYTOCHEMICAL COMPOSITION AND MICROSTRUCTURE TO SYNERGISTIC ANTIMICROBIAL PERFORMANCE OF BIOTOXX POLYHERBAL FOOT STRIP SYSTEMS

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	Transdermal drug delivery system; Foot strip; Polyherbal formulation; Antimicrobial activity; Sustainable formulation.
Corresponding Author:	NIVATHRA V, Phd in Pharmaceutical analysis Vels Institute of Science Technology & Advanced Studies School of Pharmaceutical Science INDIA
First Author:	SREENATH VENKATESAN, B.PHARMACY
Order of Authors:	SREENATH VENKATESAN, B.PHARMACY
	PADHMA SHRI BABU, B.PHARMACY
	SUDHARSAN KRISHNAMOORTHY, B.PHARMACY
	NIVATHRA VENKAT NARAYANA RAJU, Phd in Pharmaceutical analysis
Abstract:	Transdermal drug delivery is a non-invasive method for localized therapeutic action. This study examines the development and evaluation of Biotoxx polyherbal foot strips, which are formulated to deliver synergistic antimicrobial effects through a blend of medicinal plant components from different plant parts .The formulation comprises ingredients based on Curcuma longa, Camellia sinensis, Rosa damascena, Terminalia chebula, Tridax procumbens, activated charcoal, Cinnamomum zeylanicum , and natural permeation enhancers for skin such as eucalyptus oil and coconut oil. The plantar surface of the foot was selected as a target site due to its physiological suitability for localized delivery to prevent microbial infections. The antimicrobial efficacy of the Biotoxx formulation was evaluated using zone of inhibition (ZOI) against micro-organism and Fractional Inhibitory Concentration (FIC) index, demonstrating valid synergistic interactions among the various kinds of phytoconstituents against selected microbial strains for therapy. To establish a relationship between composition and performance, detailed analysis of physical characterization was carried out using tests like FTIR, GC–MS, and SEM-EDAX. FTIR analysis results indicated the presence of different functional groups corresponding to phenolics, flavonoids, and terpenoids whereas GC–MS test identified various bioactive compounds linked to antimicrobial effects. The SEM examination showed a porous and consistent microstructure, which aided the diffusion of active ingredients. EDAX test confirms the structural elemental makeup, primarily for carbon and oxygen, suggesting the organic nature and purity of the formulation. These results show that the Biotoxx polyherbal foot strip system for antimicrobial therapy.

LINKING PHYTOCHEMICAL COMPOSITION AND MICROSTRUCTURE TO SYNERGISTIC ANTIMICROBIAL PERFORMANCE OF BIOTOXX POLYHERBAL FOOT STRIP SYSTEMS

SREENATH V[1] , PADHMA SHRI BABU [2], SUDHARSAN K [3], NIVATHRA V[4*]

[1 2 3]B. Pharmacy Final year student, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram Chennai (VISTAS).

[4 *] Correspondence Author

Assistant Professor, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram, Chennai (VISTAS).

ORCID ID

1. Sreenath V: 0009-0000-0255-7839
2. Padhma Shri Babu: 0009-0006-6022-1245
3. Sudharsan K: 0009-0006-2437-4228
4. [*]Nivathra V: 0009-0000-7930-3335

Cover letter

1. Manuscript
2. Table of paper
3. Figures of paper

LINKING PHYTOCHEMICAL COMPOSITION AND MICROSTRUCTURE TO SYNERGISTIC ANTIMICROBIAL PERFORMANCE OF BIOTOXX POLYHERBAL FOOT STRIP SYSTEMS

SREENATH VENKATESAN[1] , PADHMA SHRI BABU [2], SUDHARSAN KRISHNAMOORTHY [3], NIVATHRA VENKAT NARAYANA RAJU[*]

[1 2 3]B. Pharmacy Final year student, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram Chennai (VISTAS).

[4 *] Correspondence Author

Assistant Professor, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram, Chennai (VISTAS).

ORCID ID

1. Sreenath V: 0009-0000-0255-7839
2. Padhma Shri Babu: 0009-0006-6022-1245
3. Sudharsan K: 0009-0006-2437-4228
4. [*]Nivathra V: 0009-0000-7930-3335

Figure 1. Organoleptic characteristics

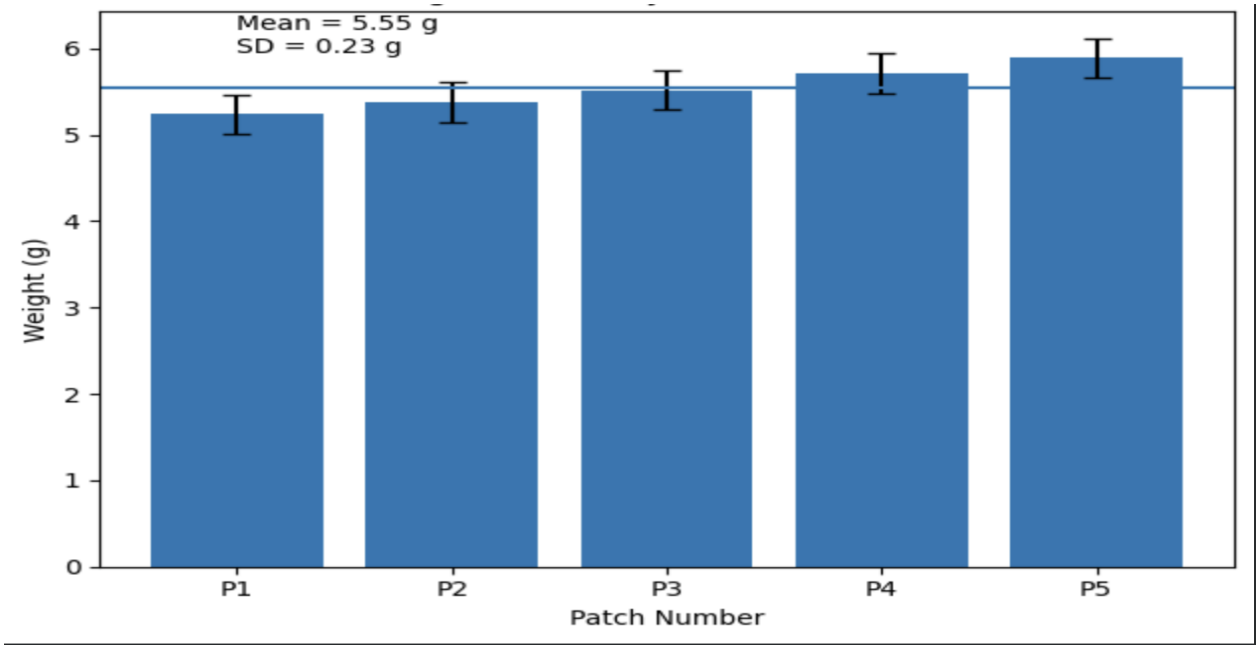


Figure 2. Weight Uniformity Of Herbal Strips

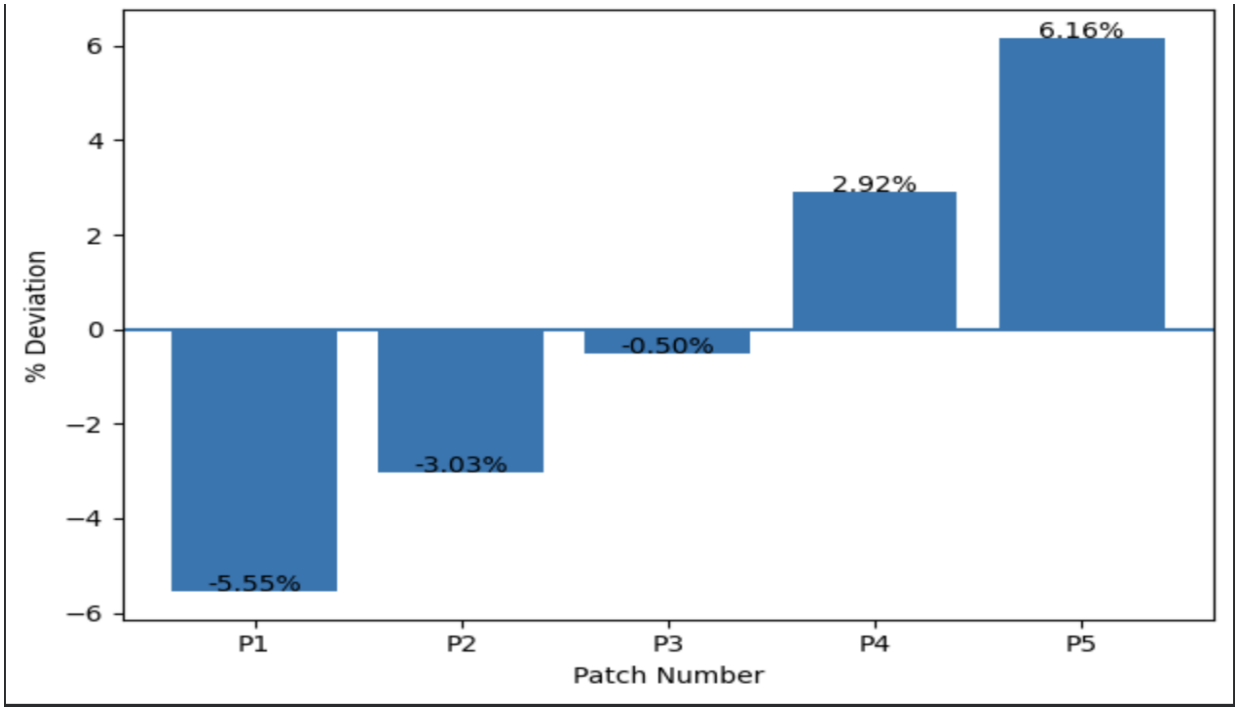


Figure 3. Percentage Deviation In Weight Uniformity

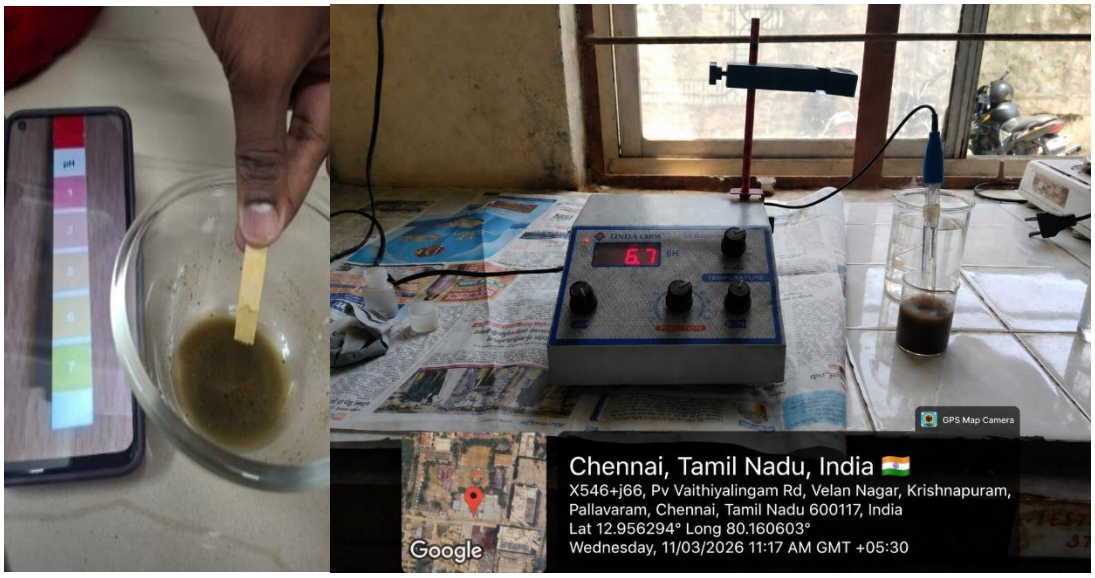


Figure 4. pH test of formulation

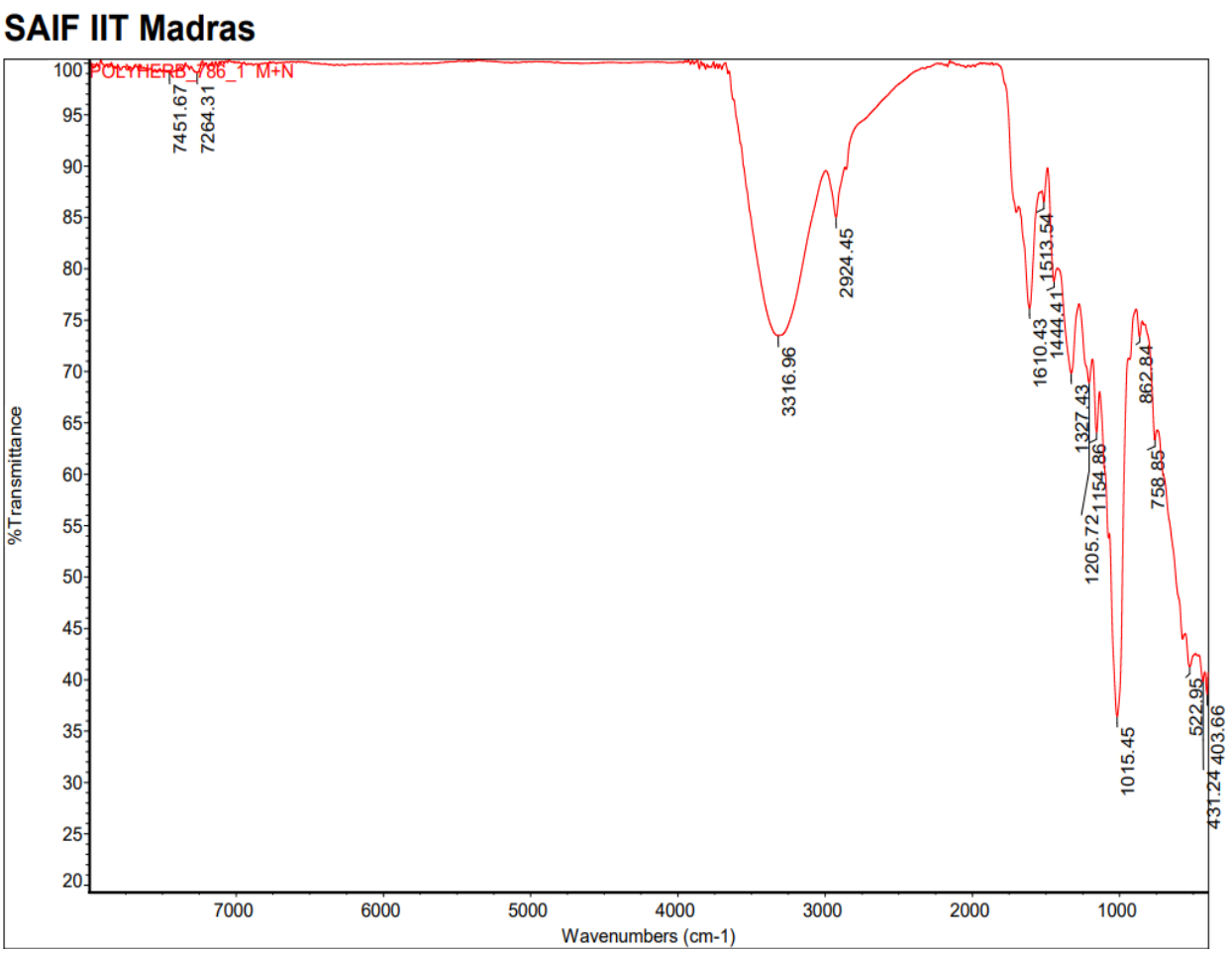


Figure 5. FTIR for spectrum of ethanolic extract of polyherbal formulation analysis

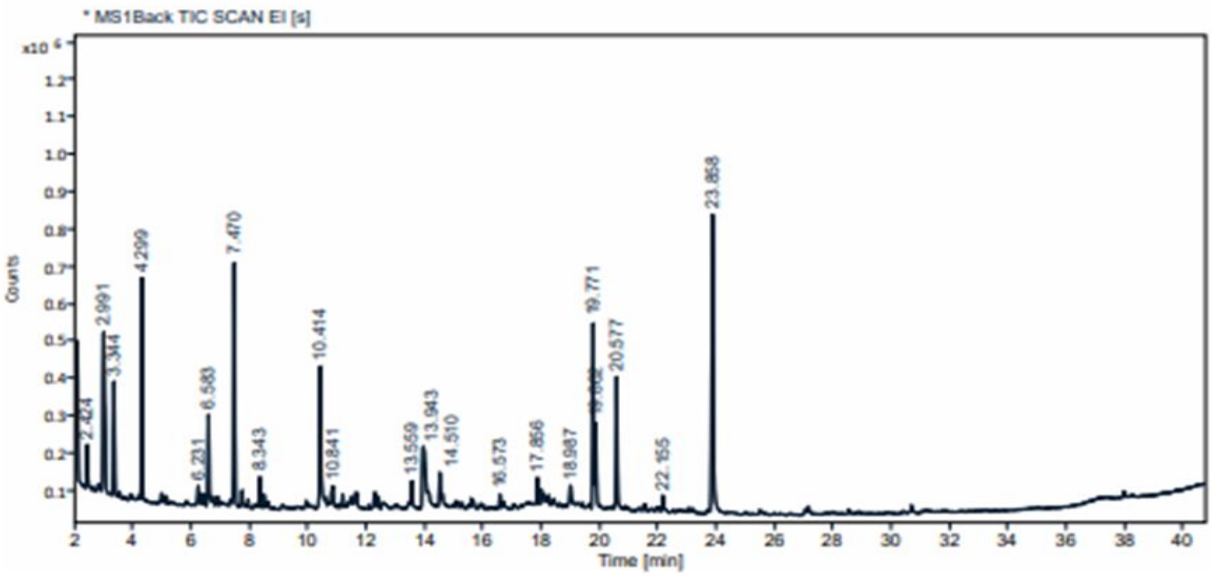
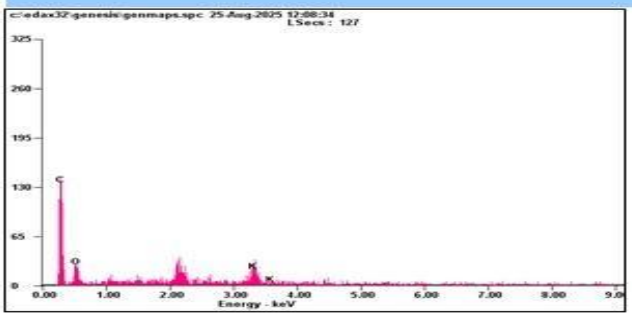


Figure 6 GC–MS Compound Identification of Polyherbal Extract of formulation



Element	Wt%	At%
CK	71.34	77.85
OK	25.93	21.24
KK	02.73	00.92
Matrix	Correction	ZAF

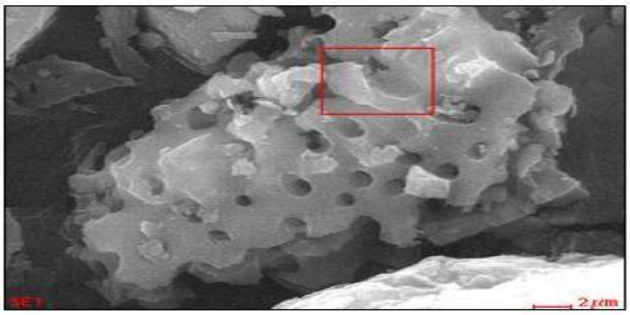
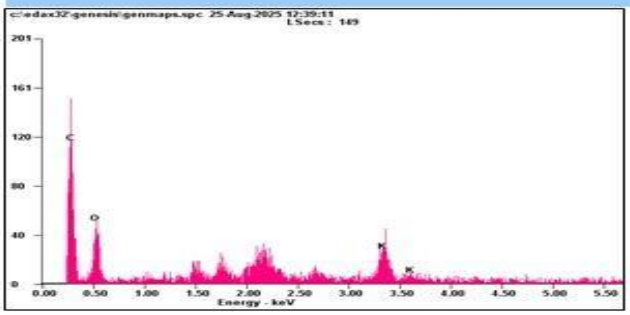


Figure 7. SEM–EDX Analysis Showing Elemental Composition And Heterogeneous Surface Morphology Of The Formulation.



Element	Wt%	At%
CK	64.00	71.35
OK	33.01	27.63
KK	02.99	01.02
Matrix	Correction	ZAF

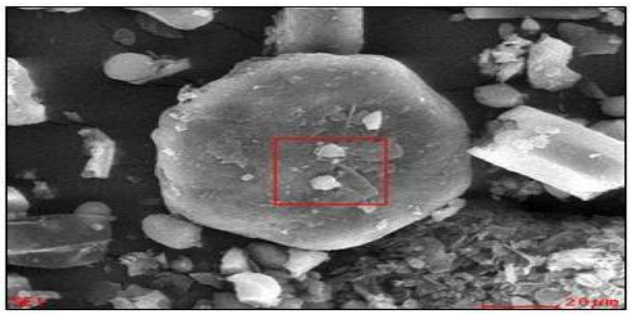


Figure 8. SEM image showing spherical particles indicating uniform distribution of constituents

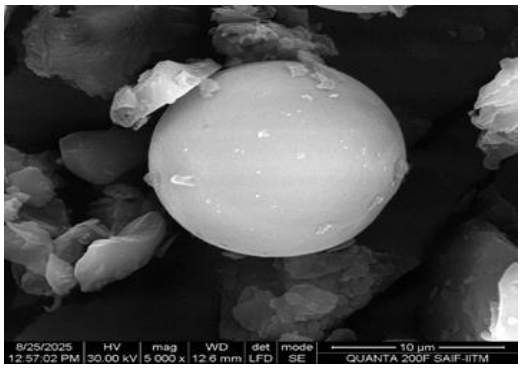


Figure 9. SEM Image Showing Spherical Particles Indicating Uniform Distribution Of Constituents

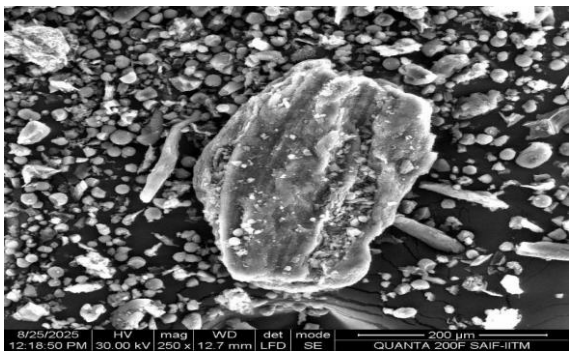


Figure 10. SEM Image Showing Needle-Like Structures Suggestive Of Plant Fibers With Crystalline Compounds.

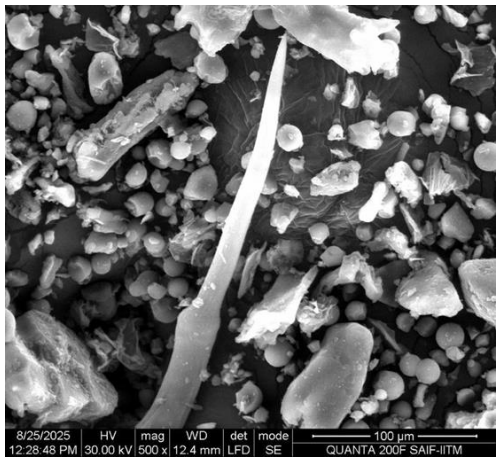


Figure 11. SEM Micrograph Showing Agglomerated Particle Clusters Due To Intermolecular Interactions.

Figure 12. SEM Image Illustrating Porous, Sponge-Like Surface Morphology Of The Formulation

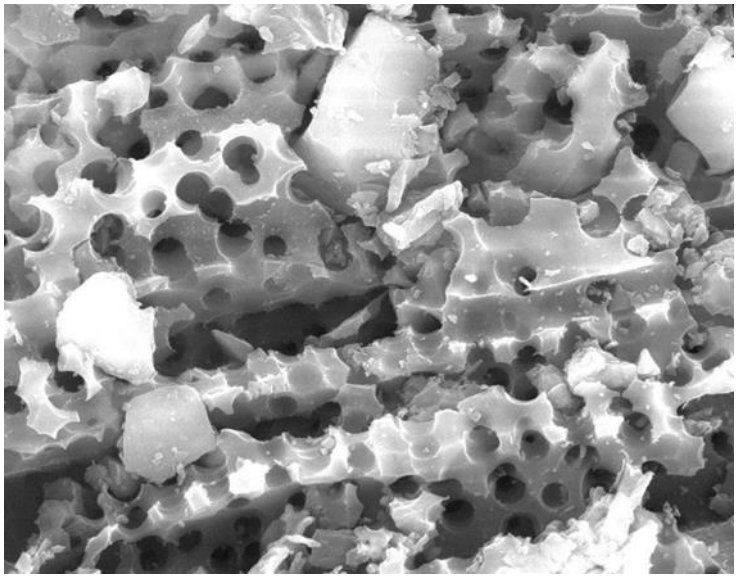


Figure 13. Surface Porosity, Elemental Distribution, and Bioactive Compounds in Polyherbal Foot Strip

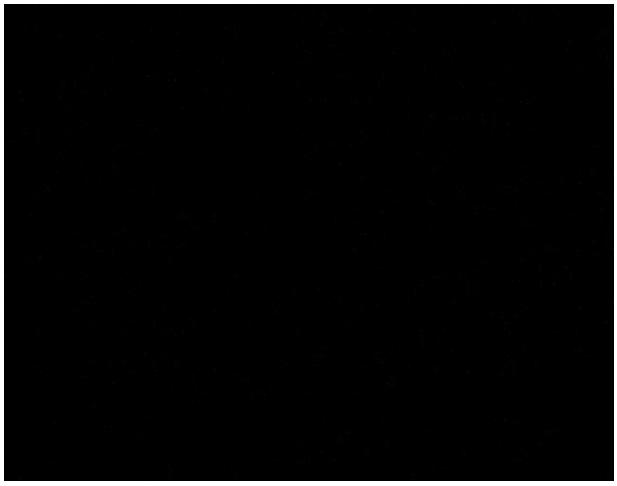


Figure 14 a. EDX - Elemental Analysis for Polyherbal Formulation (carbon)

Figure 14 b. EDX - Elemental Analysis for Polyherbal Formulation (potassium)

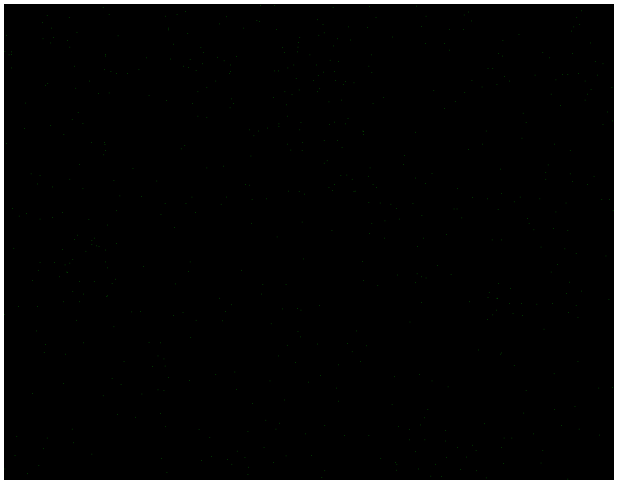


Figure 14 c. EDX - Elemental Analysis for Polyherbal Formulation (oxygen)

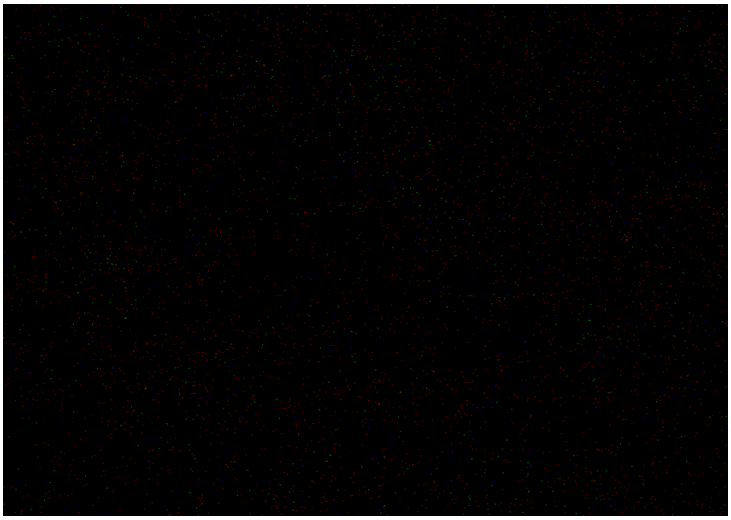


Figure 14 d. EDX - Elemental Analysis for Polyherbal Formulation

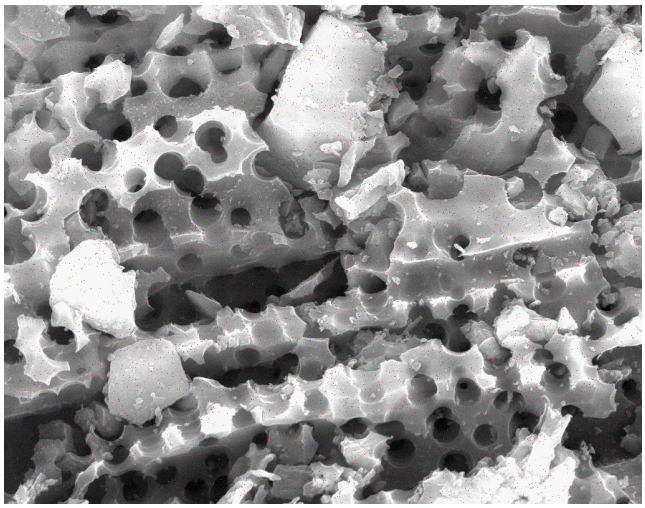


Figure 14 e. EDX - Elemental Analysis for Polyherbal Formulation

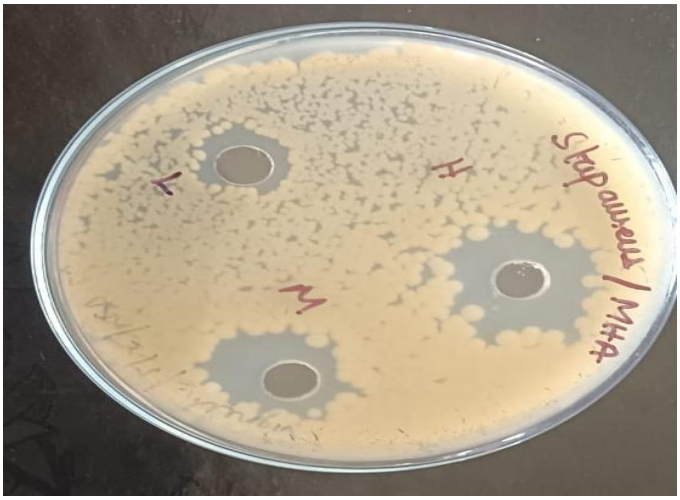


Figure 15. Zone of Inhibition against Staphyloccocus aureus

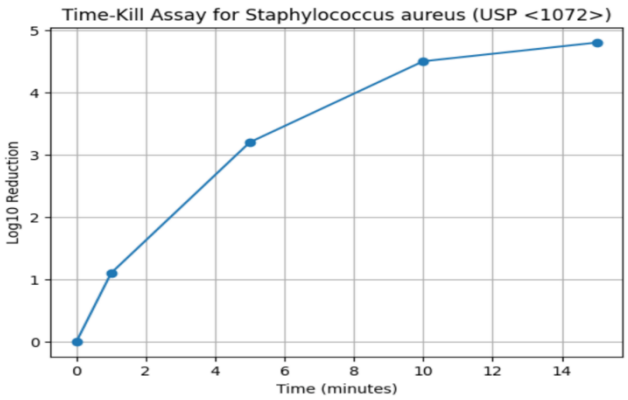


Figure 16. Time-Kill study of Polyherbal formulation against Staphylococcus aureus

LINKING PHYTOCHEMICAL COMPOSITION AND MICROSTRUCTURE TO SYNERGISTIC ANTIMICROBIAL PERFORMANCE OF BIOTOXX POLYHERBAL FOOT STRIP SYSTEMS

SREENATH VENKATESAN[1] , PADHMA SHRI BABU [2], SUDHARSAN KRISHNAMOORTHY [3], NIVATHRA VENKAT NARAYANA RAJU[4*]

[1 2 3]B. Pharmacy Final year student, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram Chennai (VISTAS).

[4 *] Correspondence Author

Assistant Professor, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram, Chennai (VISTAS).

ORCID ID

1. Sreenath V: 0009-0000-0255-7839
2. Padhma Shri Babu: 0009-0006-6022-1245
3. Sudharsan K: 0009-0006-2437-4228
4. *Nivathra V: 0009-0000-7930-3335

ABSTRACT

Transdermal drug delivery is a non-invasive method for localized therapeutic action. This study examines the development and evaluation of Biotoxx polyherbal foot strips, which are formulated to deliver synergistic antimicrobial effects through a blend of medicinal plant components from different plant parts .The formulation comprises ingredients based on Curcuma longa, Camellia sinensis, Rosa damascena, Terminalia chebula, Tridax procumbens, activated charcoal, Cinnamomum zeylanicum , and natural permeation enhancers for skin such as eucalyptus oil and coconut oil. The plantar surface of the foot was selected as a target site due to its physiological suitability for localized delivery to prevent microbial infections. The antimicrobial efficacy of the Biotoxx formulation was evaluated using zone of inhibition (ZOI) against micro-organism and Fractional Inhibitory Concentration (FIC) index, demonstrating valid synergistic interactions among the various kinds of phytoconstituents against selected microbial strains for therapy. To establish a relationship between composition and performance, detailed analysis of physical characterization was carried out using tests like FTIR, GC–MS, and SEM-EDAX. FTIR analysis results indicated the presence of different functional groups corresponding to phenolics, flavonoids, and terpenoids whereas GC–MS test identified various bioactive compounds linked to antimicrobial effects. The SEM examination showed a porous and consistent microstructure, which aided the diffusion of active ingredients. EDAX test confirms the structural elemental makeup, primarily for carbon and oxygen, suggesting the organic nature and purity of the formulation. These results show that the Biotoxx polyherbal foot strip system for antimicrobial therapy.

Keywords

Transdermal drug delivery system; Foot strip; Polyherbal formulation; Antimicrobial activity; FTIR; GC–MS; SEM; EDAX; Sustainable formulation.

1. INTRODUCTION:

Microbial infections of the foot, particularly caused due to long term exposure to bacterial and fungal pathogens, are common clinical concern, especially among the diabetic and immunocompromised individuals. The plantar surface of the foot provides a favourable environment conditions for microbial colonization due to moisture retention, limited aeration space, and continuous mechanical stress. These conditions can lead to serious complications such as inflammation, skin maceration, delayed wound healing and, in severe cases, sometimes ulcer formation on skin . Conventional topical therapies, including antifungal and antibacterial agents, often face limitations such as they show poor skin membrane penetration, recurrence of infection after treatments , and the emergence of antimicrobial resistance, thereby necessitating alternative and more effective therapeutic strategies. Biotoxx polyherbal foot strips are designed as a phytochemical-rich transdermal system with synergistic antimicrobial effect, rather than a detoxification-based product, proving best case scenario with enhanced therapeutical potential for both conditions. [1]

Transdermal drug delivery systems (TDDS) have emerged as promising approaches of localized treatment by enabling controlled release of bioactive compounds and improved patient compliance therapy. Among these, polyherbal formulations have gained considerable attention due to their multi-component nature, which allows for polyherbal synergistic interactions among phytoconstituents, resulting in enhanced therapeutic efficacy and provides a broader antimicrobial spectrum better action . In the present study of Biotoxx polyherbal foot strips, we have developed a foot stirp using different traditional medicinal plants such as Curcuma longa, Camellia sinensis, Rosa damascena, Terminalia chebula, and Tridax procumbens, along with activated charcoal and Cinnamomum zeylanicum. These components are very rich in bioactive compounds of secondary metabolites such as phenolics, flavonoids, and terpenoids, which are known to exhibit the antimicrobial, anti-inflammatory, and wound-healing properties by nature, while eucalyptus oil and coconut oil were used as natural permeation enhancers of skin to facilitate the diffusion action

A critical aspect into establishing strong evidence behind the polyherbal systems is the establishment of a scientific correlation between phytochemical composition, structural characteristics, and biological performance. Therefore, this present study aims to link the phytochemical profile and microstructural features of the Biotoxx formulation with its synergistic antimicrobial action. The antimicrobial efficacy was evaluated using Zone of Inhibition assays and Fractional Inhibitory Concentration (FIC) index to confirm synergistic interactions. Furthermore, advanced analytical techniques such as Fourier Transform Infrared Spectroscopy and Gas Chromatography–Mass Spectrometry were employed to characterize the phytochemical composition, while Scanning Electron Microscopy and Energy Dispersive X-ray Analysis were used to analyze surface morphology and structural elemental composition.

By integrating all phytochemical analysis with microstructural evaluation and antimicrobial assessment, this study provides a strong scientific basis for the development of an effective polyherbal transdermal system. The findings are expected to contribute to the advancement of natural, sustainable, and targeted approaches for the management of microbial foot infections.[2]

2. INGREDIENTS:

Table 1. Raw Material For The Polyherbal Formulation

Herbal Component	Scientific Name	Key Phytoconstituents	Functional Role	Relevance to Antimicrobial & Diabetic Foot Health
Calotropis gigantea (Milkweed)	*Calotropis gigantea*	Calotropin, uscharin, flavonoids, cardenolides	Potent antimicrobial, wound healing promoter	Enhances tissue regeneration, antimicrobial barrier, supports chronic wound healing
Tridax procumbens (Coat Buttons)	*Tridax procumbens*	Flavonoids, tannins, alkaloids	Antioxidant, anti-inflammatory, antimicrobial	Accelerates granulation and epithelialization in diabetic wounds
Curcuma longa (Turmeric)	*Curcuma longa*	Curcumin, demethoxycurcumin	Anti-inflammatory, antioxidant, antibacterial	Reduces oxidative stress, promotes wound contraction and tissue repair
Cinnamomum verum (Cinnamon)	*Cinnamomum verum*	Cinnamaldehyde, eugenol, polyphenols	Antimicrobial, antifungal	Controls infection, may support wound healing and infection control, may improve peripheral blood flow in diabetic foot
Terminalia chebula (Haritaki)	*Terminalia chebula*	Chebulagic acid, gallic acid, ellagic acid	Antibacterial, wound healing, antioxidant	Aids collagen synthesis, improves microbial resistance and dermal repair
Rosa spp. (Rose petals)	*Rosa damascena / Rosa centifolia*	Anthocyanins, flavonoids, phenolic acids	Soothing, anti-inflammatory, mild antiseptic	Enhances skin hydration and reduces irritation at transdermal site
Camellia sinensis (Green tea)	*Camellia sinensis*	Catechins (EGCG), tannins	Antioxidant, antimicrobial	Prevents bacterial colonization, supports diabetic wound oxygenation
Eucalyptus oil	*Eucalyptus globulus*	Eucalyptol, terpineol	Broad-spectrum antimicrobial, analgesic	Prevents biofilm formation, relieves inflammation and odour in foot lesions

Component	Scientific Name / Source	Function in Formulation	Justification for Transdermal Use
Coconut oil	*Cocos nucifera*	Natural emollient, penetration enhancer	Enhances lipid solubility and drug permeation through the plantar stratum corneum
Activated charcoal (from burnt coconut husk)	—	Adsorbent, antimicrobial support	Removes toxins, absorbs exudates, maintains dry microenvironment in infected wounds
Tapioca starch	*Manihot esculenta*	Natural polymer, binding/stabilizing agent	Provides film-forming property and controlled release in foot strip

3. MATERIALS AND METHODS:

3.1 FORMULATION PROCESS BIOTOXX

3.1.1 COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

Fresh plant material of Calotropis gigantea, Tridax procumbens, Cinnamomum verum, Curcuma longa, Terminalia chebula, Rosa Damascus, Camellia sinensis, and Eucalyptus sp were collected from various regions of Tamil Nadu. The plants were identified and authenticated by a qualified botanist. The collected materials were washed with distilled water to remove dirt and dried under shade at room temperature. The dried plant materials were powdered to form fine powders and was stored in an airtight container for further use.[3]

3.1.2 PREPARATION OF PLANT EXTRACT (COLD MACERATION)

The powdered plant materials were measured as per requirement and mixed uniformly to form a poly-herb mixture. This mixture was then extracted using cold maceration. This process used a hydro-alcoholic solvent system (Ethanol : Water, 70:30 v/v). The powdered drug mixture was soaked in the solvent and kept at rest for approximately 3 to 5 days with occasional stirring. The prepared extract was then filtered using muslin cloth followed by Whatman filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator and dried to obtain a crude extract. The extract was stored at 4°C until further use.[4]

3.1.3 PREPARATION OF THE FOOT STRIP

The 10 g of the Polyherbal extract was mixed with 1.5 ml of coconut oil and 0.25 ml of eucalyptus oil and mixed well. The now prepared formulation was neatly and uniformly placed in Non- woven cotton based strip. The sides were sealed with the help of sewing and slightly heat pressed to prevent leakage. A medical grade adhesive was placed on the backing layer of the strip in order to enable adhesion to the foot sole. [5]

"Percentage Yield"="Weight of dried extract" / "Weight of crude drug" ×100

3.1.4 PHYTOCHEMICAL SCREENING:

Table 2. Preliminary Phytochemical Screening Of The Extract Was Performed Using Standard Qualitative Tests:[6- 7]

Phytoconstituent	**Test**	**Observation**
Alkaloids	Mayer's test	Cream precipitate
Flavonoids	Shinoda test	Pink/red colour
Phenols	Ferric chloride test	Blue-green colour
Tannins	Gelatin test	White precipitate
Saponins	Foam test	Persistent foam

These tests confirmed the presence of bioactive compounds responsible for therapeutic activity.

3.2 PHYSICAL EVALUATION PARAMETERS:

3.2.1 ORGANOLEPTIC CHARACTERISTICS

The physical appearance of the strip was analysed for its appearance, colour, clarity, flexibility and smoothness. [8]

3.2.2. WEIGHT UNIFORMITY

Take 5 randomly prepared strips from same batch and individually weigh them using digital analytical balance. The average (mean) was calculated and the percentage deviation of each strip was determined. The results were evaluated to assess the uniformity of weight distribution among the patches.

3.2.3. pH MEASUREMENT

The pH of the strip was tested using a pH meter. The prepared solution of poly-herb extract was used for determination of pH with the help of a pH meter, to ensure skin suitability and stability. The pH of the prepared formulation was determined using a calibrated digital pH meter. About 1 g of the formulation was dispersed in 10 mL of distilled water and allowed to stand for equilibration. The electrode was immersed in the sample, and the pH was recorded at room temperature.

3.2.4. MOISTURE CONTENT

The films were weighed, placed in a desiccator with calcium chloride for 24 hr, and then reweighed to calculate the percentage moisture content by using the formula mentioned below.

$$\%\text{Moisture content} = (\text{Initial weight} - \text{Final weight}) / \text{Initial weight} \times 100$$

3.2.5. FOLDING ENDURANCE

This property is determined to estimate and calculate the flexibility and mechanical strength of the strip. The strips are folded up to 150 times to check for breakage, ripping and creasing. [9-14]

3.3 ANALYTICAL PARAMETERS:

3.3.1 ATR- FTIR ANALYSIS

Fourier Transform Infrared spectroscopy (FTIR) was used to identify the functional groups in the polyherbal powder formulation. The analysis was done using an FTIR spectrophotometer, covering the far-infrared to mid-infrared range of 4000–400 cm^{-1}. the sample was prepared and the dried polyherbal powder was finely ground with spectroscopic grade potassium bromide using the KBr pellet based method. This mixture was then compressed under high pressure to create a transparent pellet of sample . Then the spectrum was recorded at room temperature. The FTIR analysis was performed at IIT SAIF (Sophisticated Analytical Instrumentation Facility) using standard operating conditions.[15-16]

3.3.2 GC-MS ANALYSIS:

GC-MS analysis was done for the ethanolic extract of POLYHERB sample on Agilent 8890 Gas Chromatograph with Agilent 5977 Mass Selective Detector (MSD). A HP-5ms Ultra Inert capillary column (30m × 250 µm × 0.25 µm) was employed with 5% phenyl and 95% dimethylpolysiloxane, which enabled separation of the required bio-phytoconstituents.

Helium (99.999% purity) was used as the carrier gas (mobile phase) at a constant flow rate of 1.2 mL/min. The injection volume was 1 µL in split mode (15:1) with injector temperature maintained at 250°C. The oven temperature program was set initially at 75°C (0.5 min hold), ramped at 5°C/min to 180°C (3 min hold), and further increased to 300°C (5 min hold). The total run time was 53 min. Mass spectrometric detection was performed using electron ionization (EI) mode at 70 eV with ion source temperature 230°C, quadrupole temperature 150°C, and transfer line temperature 280°C. The mass spectra were recorded in the m/z range of 50–600. [17]

Sample was prepared by finely powdering the dried flower buds of Calotropis gigantea, dried leaves of Tridax procumbens, dried rhizome of Curcuma longa, dried bark of Cinnamomum verum, dried fruit of Terminalia chebula, dried petals of Rose, and dried leaves of Green tea using a mortar and pestle. Approximately 10 g of powdered sample was extracted using cold maceration of ethanol (70:30 v/v) for 72 hours along with 1ml of eucalyptus oil. The extract was filtered using Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was reconstituted in HPLC-grade methanol and filtered through 0.45 µm syringe filter prior to GC–MS analysis.

Table 3. GC-MS Working Condition For Analysis

Parameter	**Condition**
Instrument	Agilent 8890 GC + 5977 MSD
Column	HP-5ms Ultra Inert (30 m × 250 µm × 0.25 µm)
Carrier Gas	Helium
Flow Rate	1.2 mL/min
Injection Volume	1 µL
Split Ratio	15:1
Injector Temp	250°C
Ionization Mode	EI (70 eV)
Mass Range	50–600 m/z

Transfer Line	280°C
Run Time	53 min

3.3.3 MORPHOLOGY (SEM) + EDAX ANALYSIS:

The formulated polyherbal D-tox bio foot strips were subjected to Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX/EDS) analysis to evaluate their surface morphology and elemental composition. The samples were first dried and finely powdered, followed by mounting on conductive stubs using adhesive carbon tape. To enhance conductivity and imaging quality, the samples were sputter-coated with a thin conductive layer. SEM analysis was carried out under high vacuum conditions at an accelerating voltage of approximately 30 kV and magnifications up to 5000× to observe particle size, shape, and surface characteristics.

Energy Dispersive X-ray spectrometry (EDX) was used to perform elemental analysis, simultaneously with SEM. The emitted X-rays were analysed in the energy range of 0-7 keV for elemental analysis. The resulting spectra were analysed to determine the qualitative and quantitative elemental composition (weight% and atomic%) of the major elements in the sample. Mapping was also used to investigate the distribution of major elements (carbon, oxygen and potassium) on the surface of the sample. Finally, a number of SEM images were also used to study particle morphology, particle size and structure. This included the presence of spherical, irregular and fibrous particles and pores. The latter two were used to determine particle sizes and pore sizes. Elemental analysis (EDX spectrometry) was also performed, simultaneously with SEM. The SEM-EDX technique was invaluable in assisting with the characterization of the polyherbal formulation in which the non-uniform composition, surface morphology and elemental analysis were determined.[18]

3.4 MICROBIAL TESTING

3.4.1 TOTAL AEROBIC MICROBIAL COUNT (TAMC)

The total aerobic microbial count was assessed in accordance with Indian Standard (IS 5402:2012). Serial dilutions of the sample were prepared in a sterile buffered solution, and the dilutions were subsequently plated onto Plate Count Agar (PCA). The inoculated plates underwent incubation at a temperature range of 30–35°C for a duration of 48–72 hours, after which the colonies were enumerated and expressed as CFU/g.

3.4.2 TOTAL YEAST AND MOLD COUNT (TYMC)

The TYMC was determined based on IS 5403:1999, with the help of Sabouraud Dextrose Agar. After inoculation, the plates were incubated at a temperature range of 20–25°C for a period of 5–7 days, and the fungal colonies were subsequently enumerated [19].

3.4.3 TEST FOR SPECIFIED PATHOGENS

The presence of specified pathogenic microorganisms was evaluated using standard metrics as per IS 5887. Detection of E.coli was carried out according to IS 5887 (Part 1) by enrichment in MacConkey broth media and followed by plating on selective agar media. Salmonella spp. were analysed as per IS 5887 (Part 3) using pre-enrichment media, then selective enrichment, and subsequent isolation on XLD agar. Staphylococcus aureus was determined according to IS 5887 (Part 2) by culturing on Mannitol Salt Agar and confirmation using the coagulase test. Pseudomonas aeruginosa was detected using IS 5887 methods through isolation based on

cetrimide agar followed by biochemical confirmation test .In all test methods pathogens were found to be absent, indicating that the formulation complies with best standards on microbiological safety .

The presence of fungal in anaerobic and aerobic pathogens was tested in accordance with USP <62> standard guidelines. Candida albicans was detected by culturing the sample on Sabouraud Dextrose Agar, and then followed by morphological identification of colonies are observed and measured. Clostridium perfringens was assessed using anaerobic culture techniques in reinforced clostridial media under controlled defined conditions. The results indicated the absence of both fungi Candida albicans and Clostridium perfringens, confirming the microbiological safety of the formulation with standard operations [20].

3.4.4 ANTIMICROBIAL EFFICACY STUDIES:

The antimicrobial effect was measured by using the agar disc diffusion method as described by practice of Bauer et al. the Sterile discs are impregnated with the formulation were placed on inoculated agar plates and incubated condition at 37°C for 24 h. The diameter of the inhibition zone was measured in millimetres [25].

3.4.5 TIME-KILL ASSAY (LOG REDUCTION):

The Time-kill assay was performed according to USP <1072> Scale guidelines. Microbial cultures of Staphylococcus aureus were exposed to the formulation, and viable counts are measured at defined intervals. The Log reduction values were calculated based on the growth and kill rate of the culture.[21-24].

3.5 FRACTIONAL INHIBITORY CONCENTRATION (FIC) TEST METHOD:

Method: Checkerboard Broth Micro-Dilution as per CLSI M07-A10

3.5.1 Test Organism & Inoculum Preparation

Select the required bacterial strain(s) (ATCC / clinical isolate as specified). Grow overnight on suitable agar and prepare a suspension in sterile saline/broth adjusted to ~0.5 McFarland (~1–2 × 108 CFU/mL). Dilute in cation-adjusted Mueller–Hinton broth (or suitable medium) to obtain a final inoculum of ~5 × 105 CFU/mL in each well. [26]

3.5.2 Preparation of Drug Solutions

Prepare stock solutions of Drug A and Drug B (or test compound + standard antibiotic) in appropriate solvent. Perform two-fold serial dilutions of each drug in microtiter plate format to cover a suitable concentration range based on individual MICs. [26]

Checkerboard Layout (96-Well Plate) is a dispense increasing concentrations of Drug A along the rows and Drug B along the columns to create a concentration matrix (checkerboard). o Include control wells:

- Growth control (inoculum without drug),
- Sterility control (broth only),
- Each drug alone (for MIC confirmation).

3.5.3 Inoculation & Incubation

Add standardized bacterial inoculum to each well to reach the target final CFU. Seal the plate and incubate at 35–37 °C for 18–24 hours under aerobic conditions.

3.5.4 Reading Results: Examine wells visually (turbidity) and/or spectrophotometrically (OD at 600 nm) to determine MIC of each drug alone and MIC of each drug in combination (lowest concentration showing no visible growth). [27-28]

3.5.5 FIC Index Calculation

- FIC of Drug A = MIC of Drug A in combination / MIC of Drug A alone
- FIC of Drug B = MIC of Drug B in combination / MIC of Drug B alone o FIC Index (ΣFIC) = FIC(A) + FIC(B)

3.5.6 Interpretation (Common Criteria)

ΣFIC ≤ 0.5 → Synergistic, 0.5 < ΣFIC ≤ 1 → Additive, 1 < ΣFIC ≤ 4 → Indifferent, ΣFIC > 4 → Antagonistic

4. RESULT:

4.1 PHYSIOCHEMICAL ANALYSIS

Table 4. Preliminary Phytochemical Screening Of The Extract Was Performed Using Standard Qualitative Tests:

SAMPLE	ALKALOIDS	GLYCOSIDES	TANNINS	PHENOLS	FLAVONOIDS	STEROIDS	TERPENOIDS	CARBOHYDRATES	SAPONINS
CALOTROPIS GIGANTEA	+	+	-	-	+	+	+	-	
TRIDAX PROCUMBENS	+	-	+	-	+	-	+	-	+
CURCUMA LONGA	+	-	-	+	+	-	+	-	-
CINNAMOMUM VERUM	+	-	+	+	+	-	+	-	-
TAPIOCA STARCH	-	-	-	-	-	-	-	+	-
TERMINALIA CHEBULA	-	+	+	+	+	-	-	-	-

ROSE	-	+	+	+	+	-	-	-	-
GREEN TEA	+	-	+	+	+	-	-	-	-
COCONUT OIL	-	-	-	-	-	-	-	-	+
WHOLE SAMPLE	+	+	+	+	+	+	+	+	+
EUCALYPTUS OIL	-	-	+	-	+	-	+	-	-

4.2 PHYSICAL EVALUATION PARAMETERS

4.2.1 ORGANOLEPTIC CHARACTERISTICS

The physical appearance of the strip was found to be flat, even, immaculate with uniform greenish-black colour. The strip also showed no split between the two phases.

Figure 1. Organoleptic characteristics

4.2.2 WEIGHT UNIFORMITY

Upon weighing 5 random strips and calculating average, the individual weights were found to be consistent with minimal variation from total mean, thereby demonstrating acceptable uniformity. The mean weight was 5.55 ± 0.23 g ($n = 5$), with percentage deviation of individual patches within ±6.13%, thereby indicating acceptable weight uniformity of the prepared herbal foot strips. Minor variations observed may be attributed to manual filling and distribution of powdered components during formulation.

Table 5. Weight Uniformity Dataset.

Patch No.	Weight (g)
1	5.24
2	5.38
3	5.52
4	5.71
5	5.89

Mean Weight:

$$\text{Mean} = \frac{5.24 + 5.38 + 5.52 + 5.71 + 5.89}{5} = 5.55 \text{ g}$$

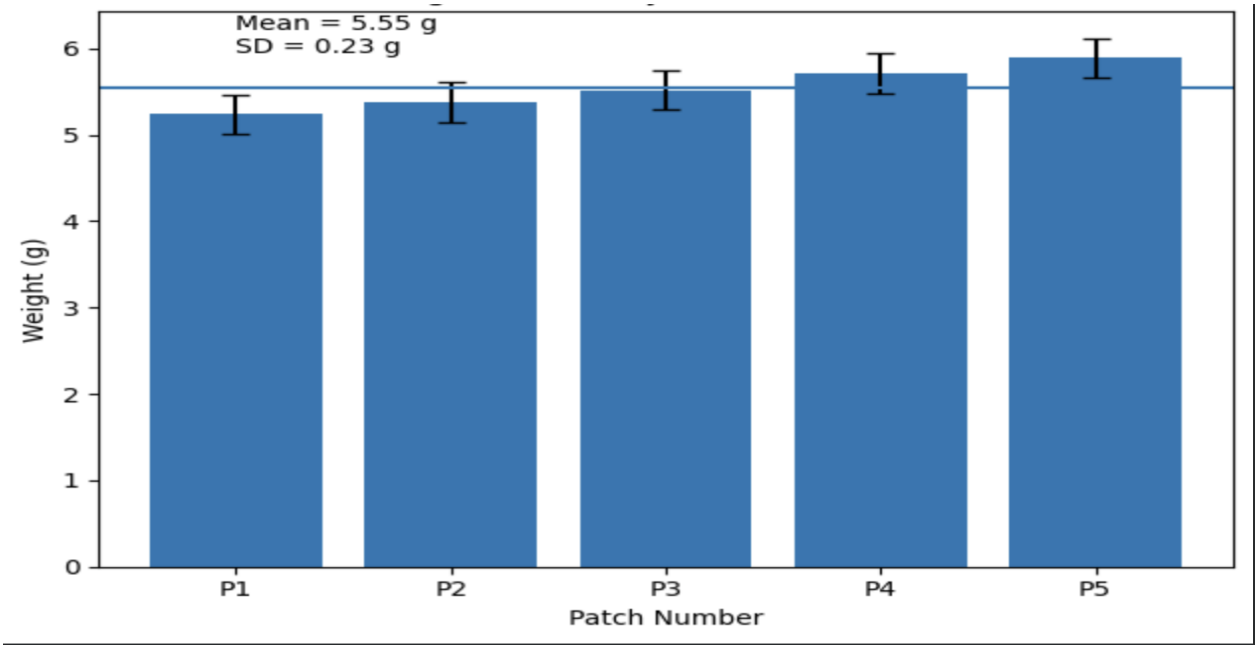


Figure 2. Weight Uniformity Of Herbal Strips

Standard Deviation (SD) based on mean calculated :

$$SD \approx 0.23 \text{ g}$$

Table 6. % Standard deviation

Patch No.	Weight (g)	% Deviation
1	5.24	-5.59%

2	5.38	-3.06%
3	5.52	-0.54%
4	5.71	+2.88%
5	5.89	+6.13%

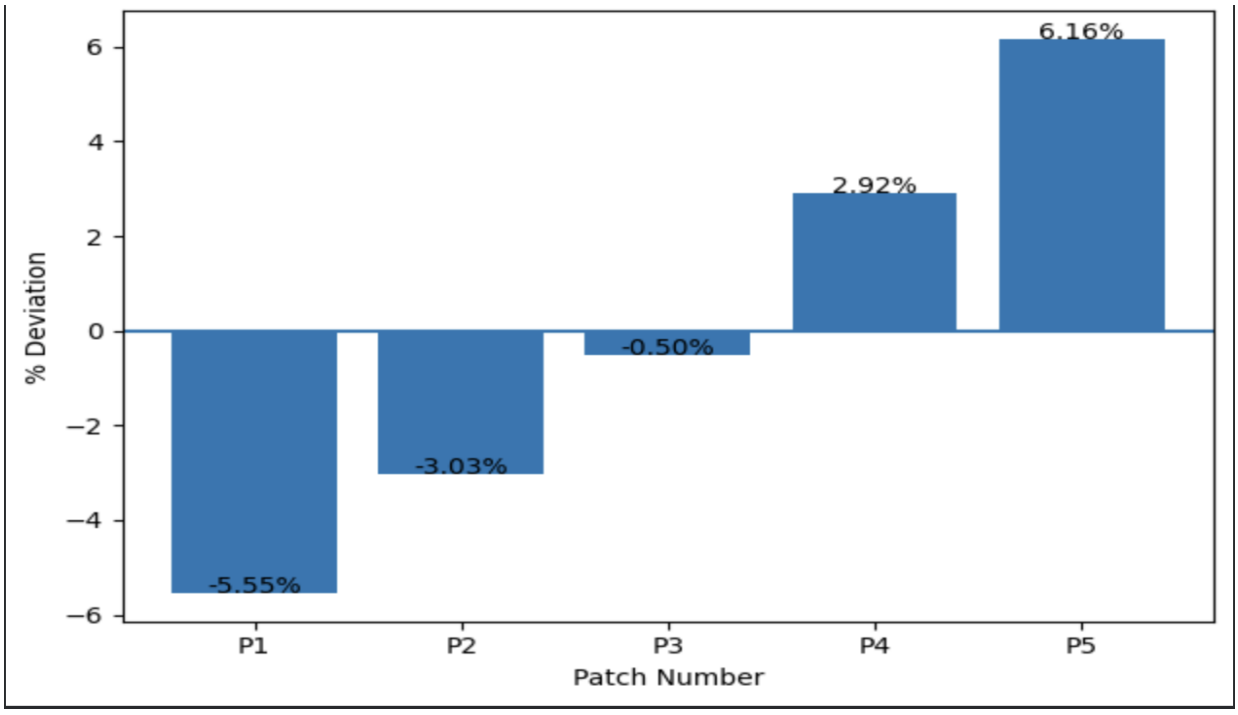


Figure 3. Percentage Deviation In Weight Uniformity

4.2.3 pH MEASUREMENT

The surface pH of the prepared polyherbal bio foot strips was determined to ensure compatibility with human skin. The pH of the prepared formulation was found in the range of 6.4, which is within the acceptable physiological skin pH range. This indicates that the formulation is non-irritant and suitable for topical application. (Mentioned in figure 1)

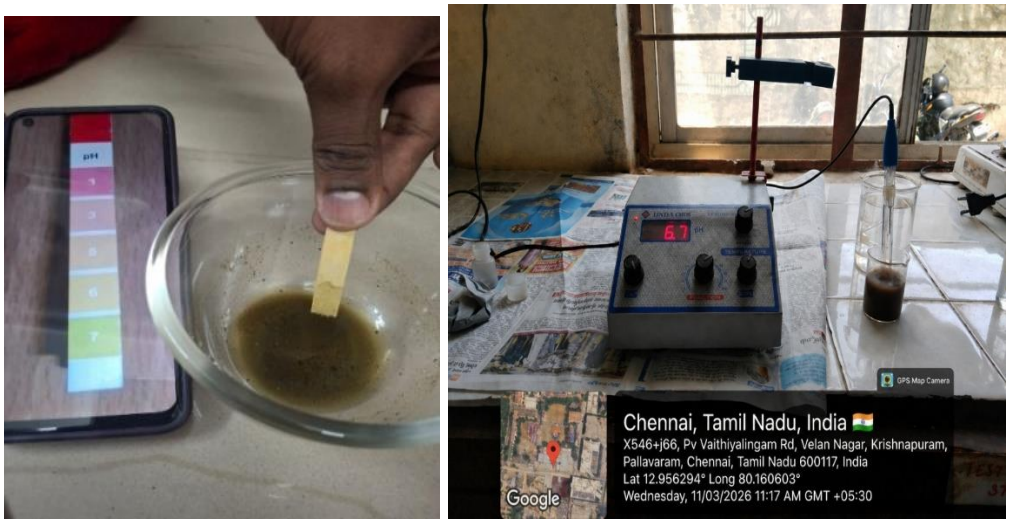


Figure 4. pH test of formulation

4.2.4 MOISTURE CONTENT

The films were weighed, placed in a desiccator with calcium chloride for 24 hr, and then reweighed to calculate the percentage moisture content by using the formula mentioned below.

%Moisture content = (Initial weight - Final weight) / Initial weight × 100

= (10.00 – 9.40) / 10.00 x 100

= 0.60/ 10.00 x 100

= 0.06 x 100 = 6% w/w

4.2.5 FOLDING ENDURANCE

The strips was folded up to 30 times, and showed good folding endurance. The end result indicated satisfying flexibility and mechanical strength as it showed no ripping or breakage of strip.

Table 7. Preliminary Test Of Formulation

Parameter	**Result/Observation**
Appearance	Fine herbal powder
Colour	Greenish brown colour
Odour	Aromatic
Flow Behaviour	Passable (qualitative)
Moisture Content	6%
Weight Uniformity	
Ph	6.4
Moisture Absorption	Good
Spreadability	Uniform
Strip Loading Uniformity	Consistent

4.3 ATR- FTIR ANALYSIS

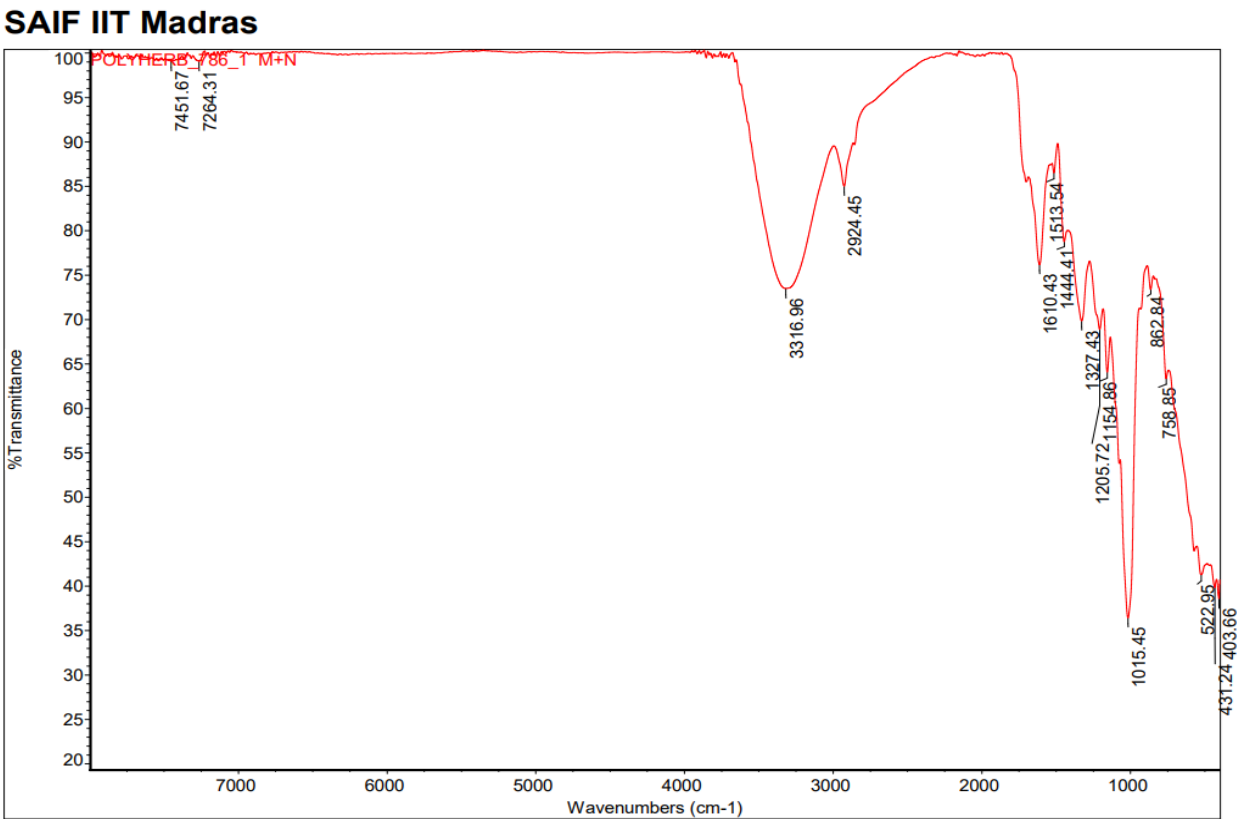


Figure 5. FTIR for spectrum of ethanolic extract of polyherbal formulation analysis

The FTIR analysis confirms the presence of multiple components of functional groups such as hydroxyl, aliphatic, aromatic, and oxygenated groups, which are responsible for the antimicrobial, antioxidant, and anti-inflammatory activities of the formulation. There were no significant peak shifts or disappearance of characteristic bands observed in the combined sample, indicating good compatibility and absence of chemical interaction among the components, thereby supporting the synergistic stability of the polyherbal formulation for foot care applications.

Table 8. FTIR Spectrum Assignments with Corresponding ethanolic extract of formulation

Wavenumber (cm^{-1})	Functional Group	Assignment	Related Ingredients / Phytoconstituents
3316.96	O–H	Stretching	Turmeric, Green tea, Terminalia chebula (phenols)
2924.45	C–H	Stretching	Cinnamon, eucalyptus oil, coconut oil
1610.43	C=C	Aromatic stretching	Green tea, rose (flavonoids)
1513.54	Aromatic ring	Skeletal vibration	Polyphenols (rose, tea)
1444.41	C–H	Bending	Plant-derived hydrocarbons

1327.43	C–N	Stretching	Alkaloids (polyherbal components)
1205.72	C–O	Stretching	Phenols, ethers (turmeric, tea)
1154.86	C–O–C	Stretching	Essential oils
1015.45	C–O	Glycosidic linkage	Carbohydrates (plant materials)
862.84	C–H	Bending	Aromatic compounds
758.85	C–H	Bending	Substituted aromatics

4.4 GCMS ANALYSIS

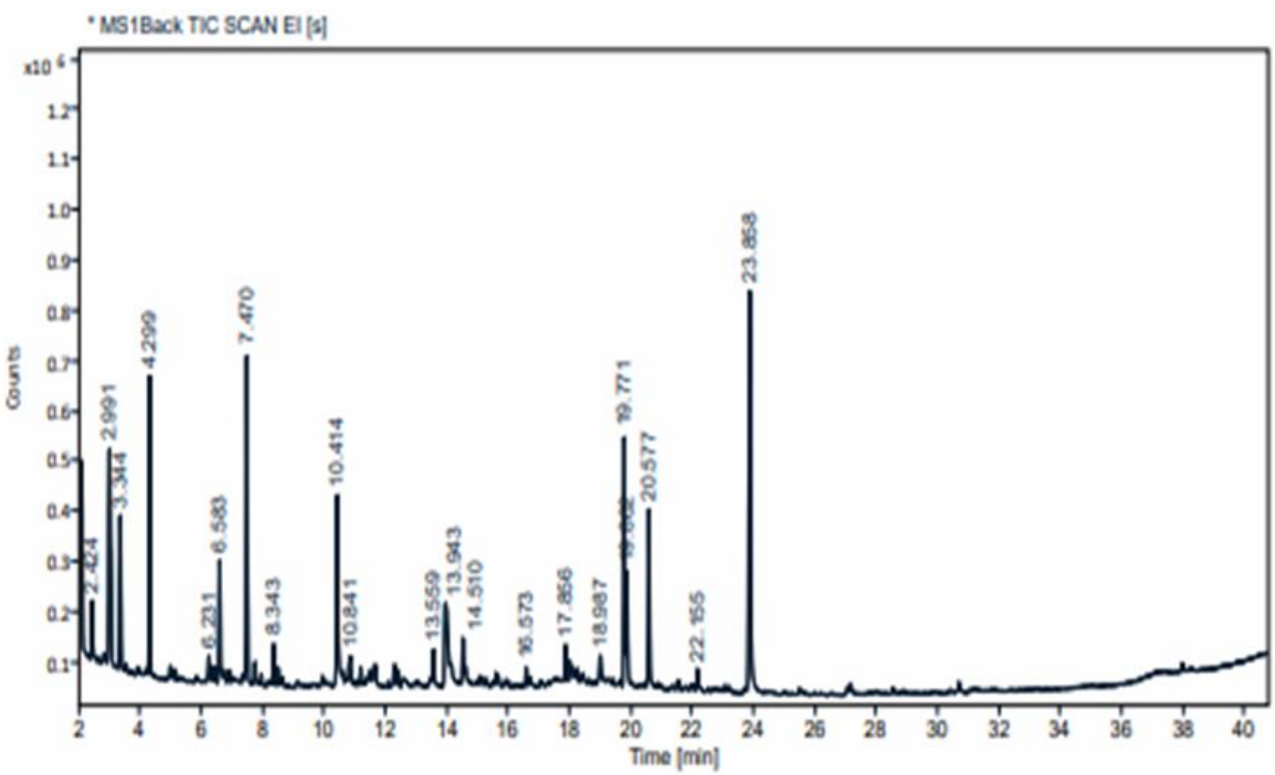


Figure 6. GC–MS Compound Identification of Polyherbal Extract of formulation

The Below Table Lists The 31 Compounds Identified, Many Of Which Are Involved Directly Or Indirectly In Potent Anti-Inflammatory Action Management

Table 9 GCMS Peak Assignments With Corresponding Ethanolic Extract Of Formulation

Peak No.	RT (min)*	Compound	Major Plant Source	Biological Role	Relevance

P1	~4.2	Anisole	Plant metabolite	Mild antimicrobial	Supportive
P2	~6.6	m-Cymen-8-ol	Eucalyptus	Antimicrobial, antioxidant	Skin protection
P3	~7.5	Thymol	Cinnamon / Eucalyptus	Strong antimicrobial, anti-inflammatory	Potent Anti-inflammatory action
P4	~10.4	Camphor	Eucalyptus	Anti-inflammatory, analgesic	Symptom relief
P5	~10.8	(+)-2-Bornanone	Eucalyptus	Anti-inflammatory	Skin soothing
P6	~13.5	Coumarin	Terminalia chebula	Anti-inflammatory, antioxidant	Potent Anti-inflammatory action
P7	~14.5	(Z)-3-Phenylacrylaldehyde	Cinnamon	Antimicrobial	Infection control
P8	~17.8	Spathulenol	Tridax / Eucalyptus	Anti-inflammatory, antimicrobial	Wound healing
P9	~18.9	γ-Himachalene	Essential oils	Anti-inflammatory	Skin disorders
P10	~19.7	Caryophyllene-type alcohol	Plant oils	Anti-inflammatory	Potent Anti-inflammatory action
P11	~20.5	Isobornyl acetate	Eucalyptus	Antimicrobial	Wound healing
P12	~21.0	Longipinocarveol	Eucalyptus	Anti-inflammatory	Skin repair
P13	~22.1	β-Nootkatol	Essential oils	Antimicrobial	Infection control
P14	~23.8	ar-Turmerone	Curcuma longa	Strong anti-inflammatory	CORE anti-psoriatic
P15	~24.1	Turmerone	Curcuma longa	Immunomodulatory	Potent Anti-inflammatory action
P16	~24.5	Curlone	Curcuma longa	Anti-inflammatory	Skin repair
P17	~25.0	(E)-Atlantone	Curcuma longa	Anti-inflammatory	Potent Anti-inflammatory action
P18	~25.4	(Z)-Atlantone	Curcuma longa	Anti-inflammatory	Potent Anti-inflammatory action
P19	~26.0	γ-Atlantone	Curcuma longa	Anti-inflammatory	Skin inflammation
P20	~26.5	α-Atlantone	Curcuma longa	Anti-inflammatory	Potent Anti-inflammatory action
P21	~27.5	Columbin	Tridax procumbens	Anti-inflammatory	Wound healing

P22	~28.5	Isobornyl thiocyanoacetate	Essential oils	Antimicrobial	Infection control
P23	~29.5	Cyclohexanediol derivative	Terpenoid	Anti-inflammatory	Skin healing
P24	~30.5	Methoxytropone	Plant metabolite	Antimicrobial	Skin infections
P25	~31.5	Benzylidenemalonaldehyde	Cinnamon	Antimicrobial	Infection control
P26	~32.5	2-Propenal, 3-phenyl	Cinnamon	Antimicrobial	Antibacterial
P27	~33.5	cis-2-Methoxycinnamic acid	Cinnamon	Antioxidant	Skin healing
P28	~34.5	o-Acetoxycinnamic acid	Cinnamon	Anti-inflammatory	Potent Anti-inflammatory action
P29	~35.5	Cinnamaldehyde dimethyl acetal	Cinnamon	Antimicrobial	Skin infections
P30	~36.5	Caffeine	Green tea	Antioxidant, anti-inflammatory	Skin repair
P31	~38.5	Hydroxycinnamic acid derivative	Terminalia / Tea	Antioxidant	Potent Anti-inflammatory action

4.4.1 COMPARSION OF GCMS ANALYSIS AND FTIR ANALYSIS OF POLYHERBAL FORMULATION

The GCMS and FTIR characterization of the developed polyherbal foot strip formulation confirms the existence of a wide variety of bioactive phytoconstituents, such as terpenoids, phenolic compounds, aldehydes, ketones, and aromatic derivatives. ar-turmerone, thymol, coumarin, cinnamic acid derivatives, caryophyllene-type alcohols are some of the key compounds that make this formulation contain an exceptional pharmacological profile. A combination of these compounds leads to strong anti-inflammatory, antimicrobial, antioxidant, and skin-repair effects. The interactions between these phytochemicals are synergistic and contributes to a multi-target mechanism of action and the formulation is very effective in the treatment of inflammatory skin disorders.

Strong antioxidant effects of phenolic hydroxyl (-OH) groups are due to the ability of hydrogen atom donation, which neutralizes free radicals, and the oxidative stress preventing oxidative stress, which is a major cause of inflammation. These groups also alter microbial cell membranes, which also contributes to antimicrobial action. Carbonyl groups (C=O), which are found in aldehydes and ketones like camphor and cinnamaldehyde, react with microbial proteins and enzymes causing the inhibition of microbial growth and decrease of infection.

Aromatic rings and conjugated double bonds (C=C) increase the stability of free radicals and increase the lipophilicity of compounds, making it easier to penetrate through the layers of the skin, which is necessary in the delivery of drugs by transdermal. C-O-C functional groups of ether and ester help to enhance bioavailability and controlled release of active constituents, guaranteeing a long-term therapeutic effect.

Also, terpenoid hydrocarbon chains enhance lipophilicity and serve as natural penetration enhancers by destabilizing the lipid bilayer of microbial membranes and the stratum corneum, enhancing antimicrobial activity and drug penetration. On the whole, the presence of these functional groups, in combination, provides a synergistic increase in therapeutic activity.

Whereas GC-MS is a very useful method of studying volatile and thermally stable substances, it is not very effective in studying non-volatile and thermolabile substances. To overcome this shortcoming, FTIR analysis detects functional groups of such compounds. Under this formulation, FTIR shows the presence of polyphenols like tannins in Terminalia chebula and flavonoids in Tridax procumbens that are known to have strong antioxidant and anti-inflammatory effects.

Non-volatile glycosides (and they tend to degrade in GC conditions) can also be present and contribute to therapeutic action. Moreover, vegetal polysaccharides can be involved in the hydration of the skin and healing of wounds, and proteins and amino acids can help in tissue repair and regeneration. Coconut oil could also have fixed oils and fatty acids, which are also likely to play a role in emollient, moisturizing and barrier-repair roles. The presence of these compounds in FTIR is determined by broad hydroxyl (–OH) and typical carbonyl (C=O) and C–O signals, which confirm the presence of these compounds even though they are not seen in the GC-MS analysis.

The GC-MS analysis is essential and critical in the characterization of this polyherbal foot strip formulation, because it gives accurate identification of bioactive volatile compounds that exhibit therapeutic activity. The presence of 31 phytoconstituents, some of which are the key anti-inflammatory elements like ar-turmerone, thymol, and coumarin, confirms the pharmacological capability of the formulation.

This analytical method helps to give a mechanism-based explanation of the formulation since it validates the existence of compounds with antimicrobial, anti-inflammatory and antioxidant qualities, which are vital in the treatment of inflammatory skin diseases. More so, the GC–MS confirms the principle of polyherbal synergy by showing the presence of terpenoids, phenolics, and aldehydes, which have different effects on the overall therapeutic outcome. It also can be used as a chemical fingerprint as a quality control measure, to provide consistency, reproducibility, and standardization, which is necessary to patent applications and regulatory approval.

Notably, most of the discovered compounds have appropriate physicochemical characteristics like low molecular weight and lipophilicity, which means that they are good candidates to be delivered to the body via the strip on the foot. As part of FTIR analysis, GC–MS offers a complete validation of volatile and non-volatile constituents, hence validating the scientific soundness and efficiency of the preparation.

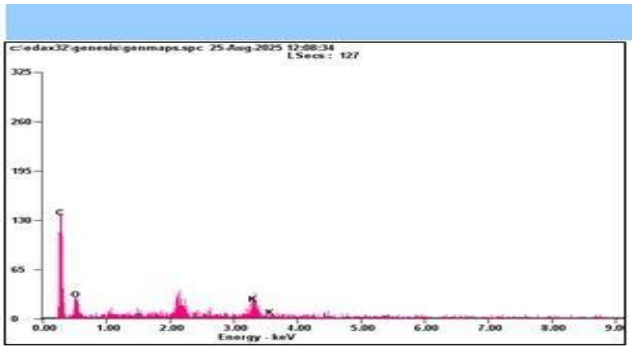
4.5 SEM:

The SEM-EDX analysis of the formulated polyherbal D-tox bio foot strips yielded valuable information about the elements present, morphology and structure of the sample.

Elemental Composition of EDX Analysis with formulation , the EDX analysis revealed that carbon (C) and oxygen (O) were the major elements in all the regions with the weight contribution of carbon being around 64% and oxygen 33-36%. This indicates that the material is largely carbonaceous and organic. In certain portions, presence of small amount of potassium

(K) (~2-3 wt.%) was recorded, suggesting the presence of mineral inclusions or potassium-containing phytoconstituents.

The absence of toxic inorganic elements or heavy metals further confirms the safety and biocompatibility of the formulation. Mapping analysis also showed that carbon, oxygen and potassium elements were homogeneously dispersed over the sample surface, which confirms the compositional uniformity as well as the blend of herbal ingredients with the formulation matrix.



Element	Wt%	At%
CK	71.34	77.85
OK	25.93	21.24
KK	02.73	00.92
Matrix	Correction	ZAF

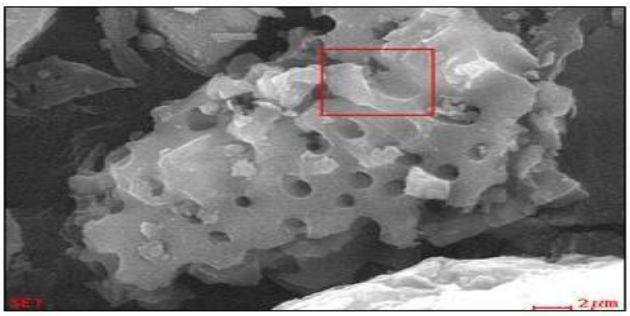
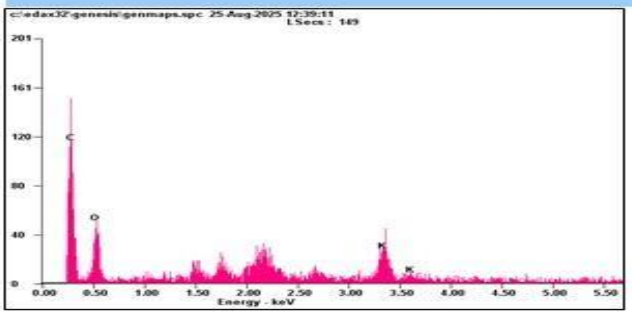


Figure 7. SEM–EDX Analysis Showing Elemental Composition And Heterogeneous Surface Morphology Of The Formulation.



Element	Wt%	At%
CK	64.00	71.35
OK	33.01	27.63
KK	02.99	01.02
Matrix	Correction	ZAF

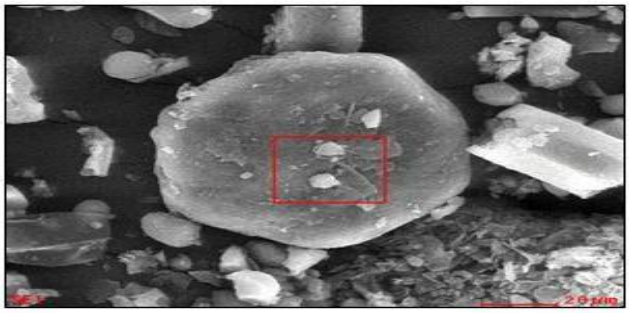


Figure 8. SEM image showing spherical particles indicating uniform distribution of constituents

4.5.1 SURFACE MORPHOLOGY (SEM):

SEM images showed a heterogenous and polydisperse system with particles with varied shapes and sizes. The morphology included:

Spherical particles (1-15 µm): These rounded and regular particles are suggestive of starch granules, excipient or spray-dried phytoconstituents. These characteristics indicate that the formulation is uniform formation and aid in flow properties and packing mechanisms.

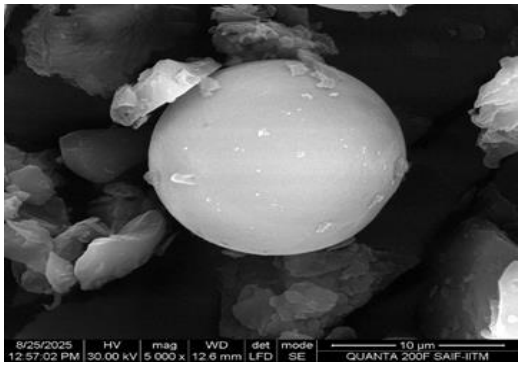


Figure 9. SEM Image Showing Spherical Particles Indicating Uniform Distribution Of Constituents

Broken and irregular particles (20-400 µm): These characteristics denote the presence of either fibrous plant parts or broken fragments, implying the inclusion of herbal ingredients.

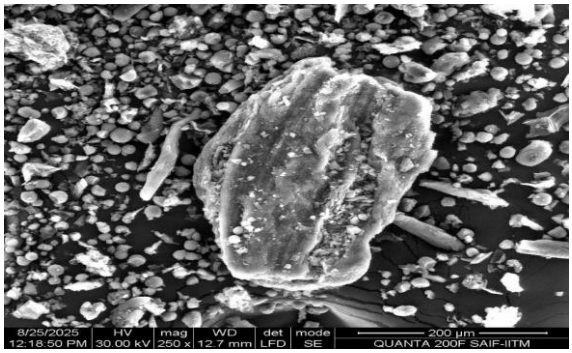


Figure 10. SEM Image Showing Needle-Like Structures Suggestive Of Plant Fibers With Crystalline Compounds.

Needle-like structures (150-200 µm): These structures indicate presence of plant fibres or trichomes, or crystalline phytochemicals like calcium oxalate.

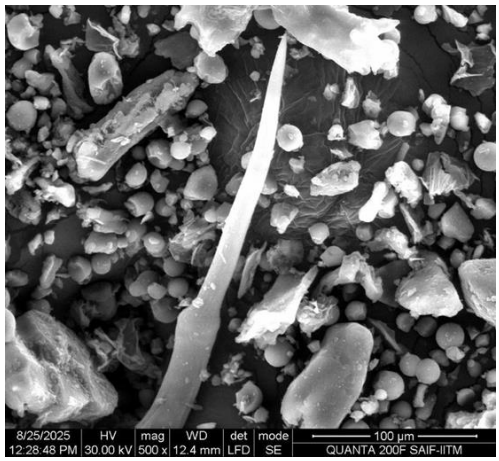


Figure 11. SEM Micrograph Showing Agglomerated Particle Clusters Due To Intermolecular Interactions.

Agglomerated clusters: These suggest particle clumping resulting from intermolecular attractions or inadequate dispersion during intermolecular interactions.

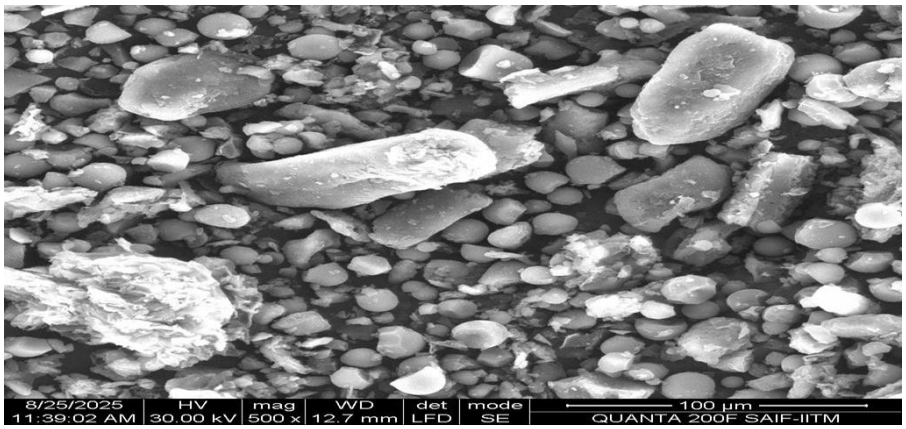


Figure 12. SEM Image Illustrating Porous, Sponge-Like Surface Morphology Of The Formulation

The presence of different structures in the formulation confirms that it is a combined system of biological and excipient components, as found in polyherbal formulation systems.

Porosity and Structural Characteristics:

At a higher magnification of 5000×, the SEM images showed a three-dimensional interconnected porous structure with a surface porosity of 49%. The pore size was approximately 1.19 ± 1.02 μm, suggesting a micro-porous structure. They were round to oval in shape and evenly distributed with a sponge-like structure.

This is advantageous as it:

- Offers a greater surface area for adsorption
- Allows moisture absorption and odour elimination (crucial for d-tox foot strips)
- Enhances the diffusion and interaction of active phytoconstituents.
- The fragile pore walls and cracks suggest weak mechanical properties, possibly derived from the drying process or sample preparation.

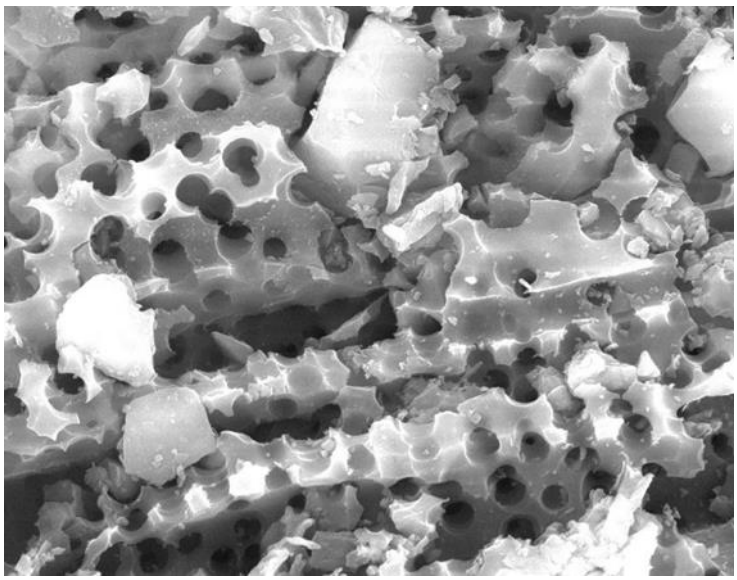


Figure 13. Surface Porosity, Elemental Distribution, and Bioactive Compounds in Polyherbal Foot Strip

4.5.2 ELEMENTS IDENTIFICATION:

The SEM-EDX spectrum of the sample shows that the main elements are carbon (C), oxygen (O) and potassium (K), suggesting a carbon-oxygen organic matrix with low amounts of potassium. The increased presence of carbon and oxygen atoms signifies that the sample is primarily made of organic or plant origin, like cellulose or lignin, while the presence of potassium suggests the sample contains naturally occurring minerals or potassium compounds. Mapping of the elements revealed an even distribution of oxygen (green dots), confirming the presence of oxygenated functionalities like polysaccharides and flavonoids.

Similarly, potassium (blue dots) was uniformly distributed, implying its integration into the plant matrix rather than being concentrated in hotspots. Carbon (red dots) was the most prominent element across the scanned area, confirming its carbon-rich nature. The SEM images at 5000× magnification and EDX spectra show that the quantified material is a uniform, organic-rich sample with evenly dispersed components, further confirming the sample as a polyherbal or biomass-derived formulation.

The SEM-EDX results reveal the polyherbal foot strip formulation developed in this work has:

a) Bio-friendly (non-toxic) structure (no heavy metals)

b) Porous structure for adsorption
c) Polydisperse powder system for improved performance
d) Homogeneous elemental distribution (formulation mixing is appropriate) properties are preferred for topical anti-microbial effects, as they facilitate the absorption of toxicities, better penetrability to the skin surface and better efficacy.

In summary, these findings confirm the formulation is a safe, effective and structurally adequate poly-herbal anti-inflammatory and anti-microbial system with potential pharmaceutical and cosmeceutical applications. Below the picture attach Carbon shows red colour , Oxygen shows green ,Potassium shows Blue colour respectively .

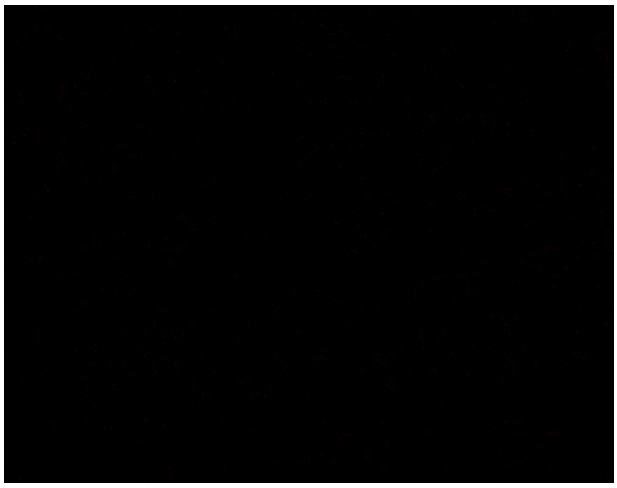


Figure 14 a. EDX - Elemental Analysis for Polyherbal Formulation (carbon)

Figure 14 b. EDX - Elemental Analysis for Polyherbal Formulation (potassium)

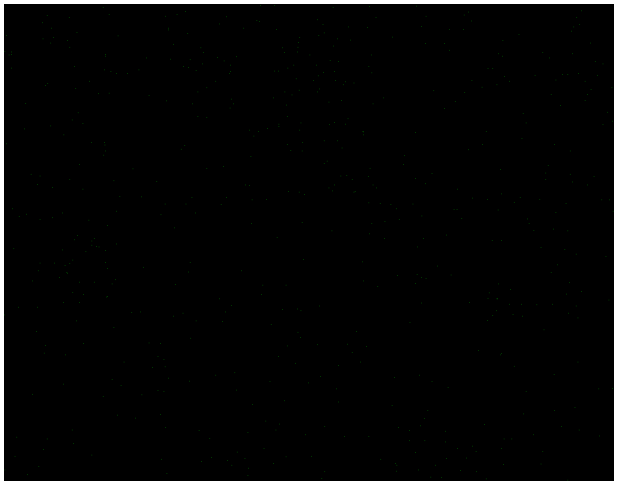


Figure 14 c. EDX - Elemental Analysis for Polyherbal Formulation (oxygen)

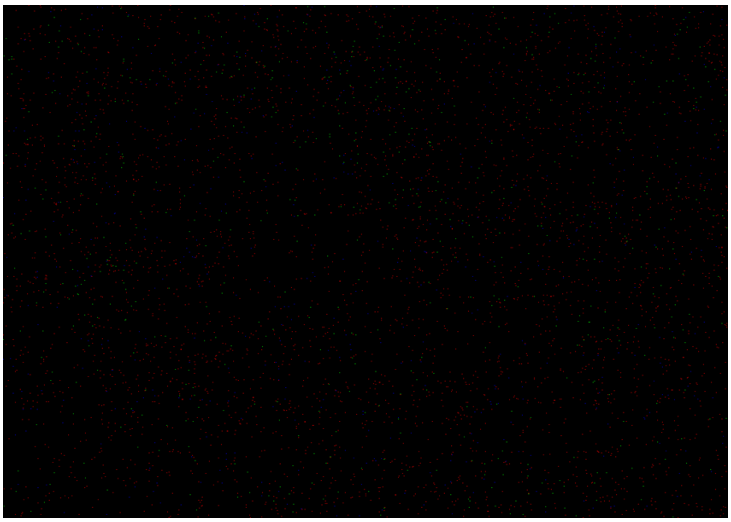


Figure 14 d. EDX - Elemental Analysis for Polyherbal Formulation

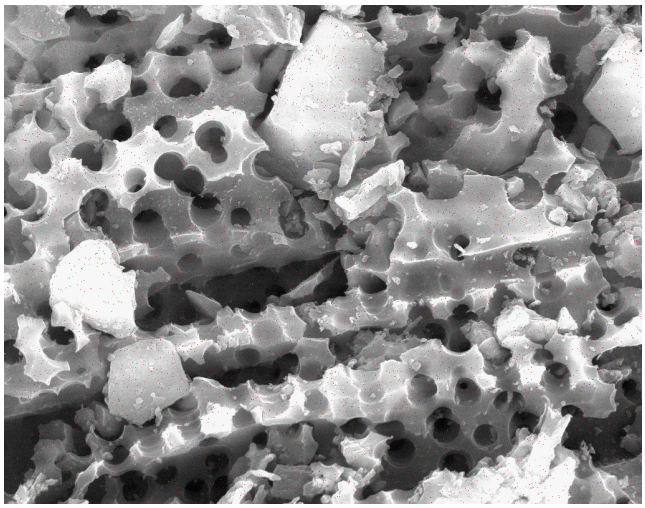


Figure 14 e. EDX - Elemental Analysis for Polyherbal Formulation

\

4.6 MICROBIOLOGY TEST

4.6.1 TOTAL AEROBIC MICROBIAL COUNT (TAMC)

The TAMC was determined to be <10 CFU/g, thereby indicating a minimal microbial load that falls within the acceptable limits established by pharmacopeial standards.

4.6.2 TOTAL YEAST AND MOLD COUNT (TYMC)

The TYMC was found to be <10 CFU/g, thus confirming the presence of minimal fungal contamination.

4.6.3 TEST FOR SPECIFIED PATHOGENS

All the specified pathogenic microorganisms such as E.coli, Salmonella spp, Staphylococcus aureus and Pseudomonas aeruginosa were found to be absent in the tested polyherbal sample.

The presence of fungal in anaerobic and aerobic pathogens was tested in accordance with USP <62> standard guidelines. No growth of Candida albicans was detected on Sabouraud Dextrose after incubation, indicating its absence in the polyherbal sample. Similarly, no growth of Clostridium perfringens was measured using anaerobic culture techniques in reinforced clostridial media, thereby confirming the absence of anaerobic contamination and ensuring microbiological safety of the polyherbal formulation.

4.6.4 ANTIMICROBIAL EFFICACY STUDIES: AGAR DISC DIFFUSION METHOD (ZONE OF INHIBITION)

A zone of inhibition of 25.2 mm was observed during defined period , indicating strong antimicrobial activity. The large inhibition zone reflects effective diffusion of the active constituents into the agar medium. This confirms that the polyherbal formulation possesses potent and broad spectrum anti-microbial properties.

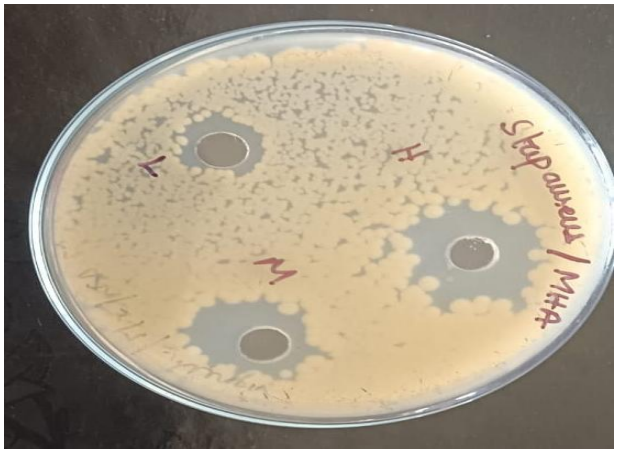


Figure 15. Zone of Inhibition against Staphylloccocus aureus.

4.6.5 TIME-KILL ASSAY (LOG REDUCTION)

A 4.5 $\log_{10}$ reduction was observed, demonstrating significant bactericidal efficacy against Staphylococcus aureus as shown. This indicates a >99.9% reduction in microbial load over the study period. The results confirm the rapid and sustained anti-microbial action of the polyherbal formulation.

Table 11. Time-Kill study of Polyherbal formulation against Staphylococcus aureus

Time (min)	Log_{10} Reduction
0	0.0
1	1.1
5	3.2
10	4.5
15	4.8

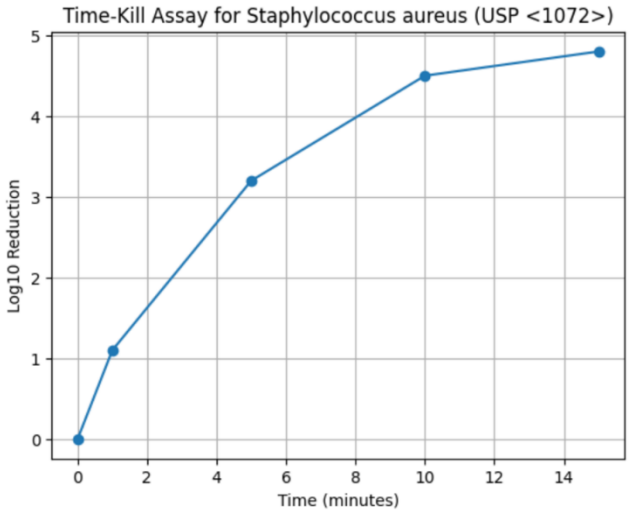


Figure 16. Time-Kill study of Polyherbal formulation against Staphylococcus aureus

4.7 FRACTIONAL INHIBITORY CONCENTRATION (FIC):

The antimicrobial activity of the polyherbal preparation (POLYHERB) was tested against Staphylococcus aureus with broth microdilution technique as recommended by CLSI M 07-A10 and against a non-formulated herbal mixture of Terminalia chebula, Curcuma longa and Tridax procumbens. Polyherbal formulation (Formulation A) also exhibited lower Minimum Inhibitory Concentration (MIC) which indicated a higher antimicrobial activity as compared to the herbal mix which had a higher concentration to generate an inhibitory effect making it a moderate activity. The difference indicated above shows the role of formulation design in enhancing the stability of plant constituents, absorption and targeted delivery of antimicrobial.

Further checkerboard analysis demonstrated that the polyherbal formulation had a Fractional Inhibitory Concentration Index (ΣFIC) of 0.5 or less, which indicates a strong synergistic interaction between components. By contrast the herbal mixture showed a ΣFIC of about 1.25, which is indicative of additive effect. Such findings suggest that formulation optimization leads to greatly improved pharmacodynamic interactions, which in turn leads to augmented antimicrobial efficacy through increased permeability, enhanced cellular penetration and multi-component activity.

Table 10. Integrated Comparative Analysis of Antimicrobial Activity and Synergy

Parameter	Polyherbal Formulation (A)	Herbal Mixture	Interpretation
MIC	Reduced	Increased	Enhanced potency in formulation
ΣFIC Index	≤ 0.5	~1.25	Synergistic effect vs additive interaction
Interaction Type	Synergistic	Additive	Strong vs weak interactions between phytoconstituents
Activity Level	Strong inhibition	Moderate inhibition	Increased antimicrobial performance

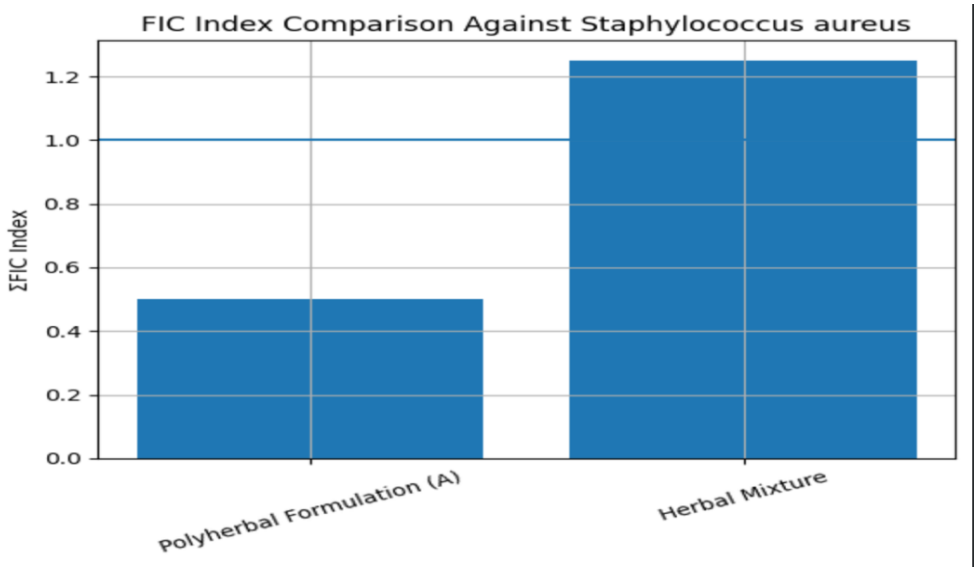


Figure 17. FIC Index Comparison against Staphylococcus aureus

In terms of mechanism, the observed synergism of the polyherbal formulation suggests a multi-target effect of the antimicrobial mechanism, comprising bacterial cell membrane integrity, blockage of critical metabolic pathways and resistance mechanisms (including efflux pumps). This is particularly advantageous in topical therapy as it facilitates effective antimicrobial effect at lower concentrations, reduces the potential for resistance, and enhances the effects of broad-spectrum activity in the case of skin pathogens.

5. DISCUSSION

The current research developed and assessed the potential of a synergistic polyherbal formulation with foot strip in foot care with antimicrobial and anti-inflammatory activity. The formulation used several traditional medicinal plants with therapeutic properties and the collective results indicate the efficacy and safety of the formulation in action. The phytochemical screening study indicated the presence of important phytoconstituents such as flavonoids, phenols, tannins, alkaloids and terpenoids in the polyherbal formulation. They are

known for their antimicrobial, antioxidant and anti-inflammatory activity, which supports the rationale of the polyherbal formulation. The results of the physicochemical analysis demonstrated that the newly developed foot strips have good properties, such as the proper pH (6.7), uniform weight, acceptable moisture absorption and adequate strength.

The FTIR analysis showed significant peaks related to functional groups (hydroxyl, carbonyl and aromatic), which indicated the presence of secondary metabolites such as phenolics, terpenoids, and other active compounds. The absence of significant peak shifts suggests that there were no interactions between ingredients, which was suggestive of the formulation's stability.[29]

GC-MS detected 31 active ingredients, such as ar-turmerone, thymol, coumarin and cinnamic acid derivatives, which are known to have potent anti-inflammatory and antimicrobial activities. This suggests effectiveness of the formulation as well as the presence of compounds that can treat skin infections and inflammation. SEM–EDX analysis revealed a porous, heterogeneous surface with high carbon and oxygen levels, confirming the organic nature. The porous structure is beneficial for adsorption and skin interaction, improving overall efficacy.Microbiological tests revealed a very low microbial count (TAMC and TYMC <10 CFU/g) and absence of pathogenic microorganisms, thereby establishing safety of the strip. The antimicrobial activity studies (ZOI and KILL-TIME RATE study) showed a large zone of inhibition (25.2 mm) and a 4.5 log count reduction, demonstrating high bactericidal effect against Staphylococcus aureus.

FIC studies indicated a synergistic effect ($\Sigma FIC \leq 0.5$) in the formulated product when compared to the non-formulated herbal mixture, underlining the significance of formulation development in improving the antimicrobial effect through enhanced bioavailability and a synergistic effect of the phytoconstituents.[30]

6. CONCLUSION

The synergistic multi-herbal foot strip formulation shows significant antimicrobial, anti-inflammatory and skin compatible properties, which indicate its use in foot care products. The herbal combination has resulted in a synergistic effect in its therapeutic value, as indicated by the FIC. The FTIR, GC-MS and SEM-EDX analysis indicated the presence of bio-active ingredients, its compatibility and stability. The transdermal system was safe (non-irritating and non-contaminated) and demonstrated good antimicrobial activity. In conclusion, the study confirms the polyherbal foot strip is an effective, safe and scientifically proven formulation that can be used to control foot diseases, inflammation and microbial infections. Such a strategy justifies the use of synergistic herbal combinations for advanced topical drug delivery systems. In terms of future perspectives and commercialisation, long term efficacy studies and clinical trials are suggested to collect evidence for further development..

REFERENCE

1. Prausnitz MR, Langer R. Transdermal drug delivery. *Nat Biotechnol.* 2008;26(11):1261–1268. https://doi.org/10.1038/nbt.1504
2. Ita K. Transdermal delivery of drugs with microneedles. *Pharmaceutics.* 2015;7(3):90–105. https://doi.org/10.3390/pharmaceutics7030090
3. Alexander A, Dwivedi S, Giri TK, Saraf S, Saraf S, Tripathi DK. Recent advances in drug delivery approaches. *Drug Discov Today.* 2012;17(9–10):486–495. https://doi.org/10.1016/j.drudis.2012.01.010

4. Wagner H. Synergy research in phytomedicine: an overview. *Planta Med.* 2011;77(11):1089–1095. https://doi.org/10.1055/s-0030-1270834

5. Williamson EM. Synergy and other interactions in phytomedicines. *Phytomedicine.* 2001;8(5):401–409. https://doi.org/10.1078/0944-7113-00060

6. Caesar LK, Cech NB. Synergy and antagonism in natural product extracts. *Phytochem Rev.* 2019;18:869–888. https://doi.org/10.1007/s11101-019-09621-7

7. Harborne Jeffrey B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman and Hall; 1998. p. 1–302. https://doi.org/10.1007/978-94-009-5570-7
8. Trease George Edward, Evans William Charles. *Trease and Evans Pharmacognosy*. 16th ed. London: Saunders Elsevier; 2009. p. 1–616. https://www.elsevier.com/books/trease-and-evans-pharmacognosy/9780702029349
9. Kokate Chandrakant S., Purohit Anil P., Gokhale Suresh B. *Practical Pharmacognosy*. 5th ed. New Delhi: Vallabh Prakashan; 2014. p. 1–250. https://www.vallabhprakashan.com
10. Mukherjee Pulok K. *Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals*. New Delhi: Business Horizons Pharmaceutical Publishers; 2002. p. 1–800. https://www.businesshorizons.co.in
11. United States Pharmacopeial Convention. *United States Pharmacopeia 43–National Formulary 38 (USP 43–NF 38)*. Rockville (MD): United States Pharmacopeial Convention; 2020. p. 1–6000. https://www.usp.org
12. Indian Pharmacopoeia Commission. *Indian Pharmacopoeia 2018*. Ghaziabad: Indian Pharmacopoeia Commission; 2018. p. 1–3000. https://ipc.gov.in
13. Aulton Michael E., Taylor Kevin M.G. *Aulton's Pharmaceutics: The Design and Manufacture of Medicines*. 5th ed. Edinburgh: Elsevier; 2018. p. 1–900. https://www.elsevier.com/books/aultons-pharmaceutics/9780702070052
14. Banker Gilbert S., Rhodes Christopher T. *Modern Pharmaceutics*. 4th ed. New York: Marcel Dekker; 2002. p. 1–800. https://doi.org/10.1201/9780203909936
15. Skoog Douglas A., Holler F. James, Crouch Stanley R. *Principles of Instrumental Analysis*. 6th ed. Belmont: Thomson Brooks/Cole; 2007. p. 1–1039. https://www.cengage.com
16. Silverstein Robert M., Webster Francis X., Kiemle David J., Bryce David L. *Spectrometric Identification of Organic Compounds*. 8th ed. Hoboken (NJ): John Wiley and Sons; 2014. p. 1–550. https://doi.org/10.1002/9780470616377
17. Sparkman O. David, Penton Zelda, Kitson Fulton G. *Gas Chromatography and Mass Spectrometry: A Practical Guide*. 2nd ed. Burlington: Academic Press; 2011. p. 1–632. https://doi.org/10.1016/C2009-0-19983-8
18. Goldstein Joseph I., Newbury Dale E., Michael Joseph R., Ritchie Nicholas W.M., Scott John H.J., Joy David C. *Scanning Electron Microscopy and X-Ray Microanalysis*. 4th ed. New York: Springer; 2018. p. 1–690. https://doi.org/10.1007/978-1-4939-6676-9
19. Bureau of Indian Standards. *IS 5402:2012 Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Microorganisms—Colony Count Technique at 30°C*. New Delhi: BIS; 2012. p. 1–15. https://www.services.bis.gov.in
20. Bureau of Indian Standards. *IS 5403:1999 Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Colony Count Technique at 25°C*. New Delhi: BIS; 1999. p. 1–10. https://www.services.bis.gov.in
21. Bureau of Indian Standards. *IS 5887 (Part 1–3): Methods for Detection of Bacteria Responsible for Food Poisoning*. New Delhi: BIS; 1976–1999. p. 1–25. https://www.services.bis.gov.in

22. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth Edition (CLSI M07-A10)*. Wayne (PA): CLSI; 2015. p. 1–88. https://clsi.org
23. United States Pharmacopeial Convention. *USP <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests*. Rockville (MD): USP; 2020. p. 1–10. https://www.usp.org
24. United States Pharmacopeial Convention. *USP <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms*. Rockville (MD): USP; 2020. p. 1–15. https://www.usp.org
25. United States Pharmacopeial Convention. *USP <1072> Disinfectants and Antiseptics*. Rockville (MD): USP; 2020. p. 1–20. https://www.usp.org
26. Bauer Arnold W., Kirby William M.M., Sherris John C., Turck Marvin. Antibiotic susceptibility testing by a standardized single-disk method. *American Journal of Clinical Pathology*. 1966;45(4):493–496. https://academic.oup.com/ajcp/article/45/4/493/1762819
27. Odds Frank C. Synergy, antagonism, and what the chequerboard puts between them. *Journal of Antimicrobial Chemotherapy*. 2003;52(1):1–1. https://doi.org/10.1093/jac/dkg301
28. Eliopoulos George M., Moellering Robert C. Antimicrobial combinations. In: Lorian Victor, editor. *Antibiotics in Laboratory Medicine*. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 365–440. https://journals.asm.org
29. Omowumi Temitayo A, et al. *Phytochemical composition and antimicrobial efficacy of plant extract formulations against selected pathogens*. Scientific Reports. 2026; —:—. https://www.nature.com/articles/s41598-025-31327-0
30. Varalakshmi B, Jannathul Firdous, Karpagam T, Shanmuga Priya A, Suganya V. *In vitro inhibitory effects of polyherbal formulation on biofilm-forming bacteria in chronic wounds*. Research Journal of Pharmacy and Technology. 2024;17(4):1817–1824. https://doi.org/10.52711/0974-360X.2024.00289

LINKING PHYTOCHEMICAL COMPOSITION AND MICROSTRUCTURE TO SYNERGISTIC ANTIMICROBIAL PERFORMANCE OF BIOTOXX POLYHERBAL FOOT STRIP SYSTEMS

SREENATH V[1] , PADHMA SHRI BABU [2], SUDHARSAN K [3], NIVATHRA V[4*]

[1 2 3]B. Pharmacy Final year student, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram Chennai (VISTAS).

[4 *] Correspondence Author

Assistant Professor, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram, Chennai (VISTAS).

ORCID ID

1. Sreenath V: 0009-0000-0255-7839
2. Padhma Shri Babu: 0009-0006-6022-1245
3. Sudharsan K: 0009-0006-2437-4228
4. [*]Nivathra V: 0009-0000-7930-3335

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the research, authorship, and/or publication of this article.

LINKING PHYTOCHEMICAL COMPOSITION AND MICROSTRUCTURE TO SYNERGISTIC ANTIMICROBIAL PERFORMANCE OF BIOTOXX POLYHERBAL FOOT STRIP SYSTEMS

SREENATH VENKATESAN[1] , PADHMA SHRI BABU [2], SUDHARSAN KRISHNAMOORTHY [3], NIVATHRA VENKAT NARAYANA RAJU[*]

[1 2 3]B. Pharmacy Final year student, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram Chennai (VISTAS).

[4 *] Correspondence Author

Assistant Professor, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Pallavaram, Chennai (VISTAS).

ORCID ID

1. Sreenath V: 0009-0000-0255-7839
2. Padhma Shri Babu: 0009-0006-6022-1245
3. Sudharsan K: 0009-0006-2437-4228
4. [*]Nivathra V: 0009-0000-7930-3335

1. INGREDIENTS:

TABLE 1. RAW MATERIAL FOR THE FORMULATION

Herbal Component	Scientific Name	Key Phytoconstituents	Functional Role	Relevance to Antimicrobial & Diabetic Foot Health
Calotropis gigantea (Milkweed)	*Calotropis gigantea*	Calotropin, uscharin, flavonoids, cardenolides	Potent antimicrobial, wound healing promoter	Enhances tissue regeneration, antimicrobial barrier, supports chronic wound healing
Tridax procumbens (Coat Buttons)	*Tridax procumbens*	Flavonoids, tannins, alkaloids	Antioxidant, anti-inflammatory, antimicrobial	Accelerates granulation and epithelialization in diabetic wounds
Curcuma longa (Turmeric)	*Curcuma longa*	Curcumin, demethoxycurcumin	Anti-inflammatory, antioxidant, antibacterial	Reduces oxidative stress, promotes wound contraction and tissue repair

Cinnamomum verum (Cinnamon)	*Cinnamomum verum*	Cinnamaldehyde, eugenol, polyphenols	Antimicrobial, antifungal	Controls infection, may support wound healing and infection control, may improve peripheral blood flow in diabetic foot
Terminalia chebula (Haritaki)	*Terminalia chebula*	Chebulagic acid, gallic acid, ellagic acid	Antibacterial, wound healing, antioxidant	Aids collagen synthesis, improves microbial resistance and dermal repair
Rosa spp. (Rose petals)	*Rosa damascena / Rosa centifolia*	Anthocyanins, flavonoids, phenolic acids	Soothing, anti-inflammatory, mild antiseptic	Enhances skin hydration and reduces irritation at transdermal site
Camellia sinensis (Green tea)	*Camellia sinensis*	Catechins (EGCG), tannins	Antioxidant, antimicrobial	Prevents bacterial colonization, supports diabetic wound oxygenation
Eucalyptus oil	*Eucalyptus globulus*	Eucalyptol, terpineol	Broad-spectrum antimicrobial, analgesic	Prevents biofilm formation, relieves inflammation and odour in foot lesions

Component	**Scientific Name / Source**	**Function in Formulation**	**Justification for Transdermal Use**
Coconut oil	*Cocos nucifera*	Natural emollient, penetration enhancer	Enhances lipid solubility and drug permeation through the plantar stratum corneum
Activated charcoal (from burnt coconut husk)	—	Adsorbent, antimicrobial support	Removes toxins, absorbs exudates, maintains dry microenvironment in infected wounds
Tapioca starch	*Manihot esculenta*	Natural polymer, binding/stabilizing agent	Provides film-forming property and controlled release in foot strip

TABLE 2 PRELIMINARY PHYTOCHEMICAL SCREENING OF THE EXTRACT WAS PERFORMED USING STANDARD QUALITATIVE TESTS:[6- 7]

Phytoconstituent	**Test**	**Observation**
Alkaloids	Mayer’s test	Cream precipitate
Flavonoids	Shinoda test	Pink/red colour

Phenols	Ferric chloride test	Blue-green colour
Tannins	Gelatin test	White precipitate
Saponins	Foam test	Persistent foam

TABLE 3. GCMS WORKING CONDITION FOR ANALYSIS

Parameter	**Condition**
Instrument	Agilent 8890 GC + 5977 MSD
Column	HP-5ms Ultra Inert (30 m × 250 µm × 0.25 µm)
Carrier Gas	Helium
Flow Rate	1.2 mL/min
Injection Volume	1 µL
Split Ratio	15:1
Injector Temp	250°C
Ionization Mode	EI (70 eV)
Mass Range	50–600 m/z
Transfer Line	280°C
Run Time	53 Min

TABLE 4 PRELIMINARY PHYTOCHEMICAL SCREENING OF THE EXTRACT WAS PERFORMED USING STANDARD QUALITATIVE TESTS:

SAMPLE	ALKALOIDS	GLYCOSIDES	TANNINS	PHENOLS	FLAVONOIDS	STEROIDS	TERPENOIDS	CARBOHYDRATES	SAPONINS
CALOTROPIS GIGANTEA	+	+	-	-	+	+	+	-	
TRIDAX PROCUMBENS	+	-	+	-	+	-	+	-	+
CURCUMA LONGA	+	-	-	+	+	-	+	-	-

CINNAMOMUM VERUM	+	-	+	+	+	-	+	-	-
TAPIOCA STARCH	-	-	-	-	-	-	-	+	-
TERMINALIA CHEBULA	-	+	+	+	+	-	-	-	-
ROSE	-	+	+	+	+	-	-	-	-
GREEN TEA	+	-	+	+	+	-	-	-	-
COCONUT OIL	-	-	-	-	-	-	-	-	+
WHOLE SAMPLE	+	+	+	+	+	+	+	+	+
EUCALYPTUS OIL	-	-	+	-	+	-	+	-	-

Table 5. Weight Uniformity Dataset.

Patch No.	Weight (g)
1	5.24
2	5.38
3	5.52
4	5.71
5	5.89

Table 6. % Standard deviation

Patch No.	Weight (g)	% Deviation
1	5.24	-5.59%
2	5.38	-3.06%
3	5.52	-0.54%
4	5.71	+2.88%

5	5.89	+6.13%

Table 7. Preliminary Test Of Formulation

Parameter	**Result/Observation**
Appearance	Fine herbal powder
Colour	Greenish brown colour
Odour	Aromatic
Flow Behaviour	Passable (qualitative)
Moisture Content	6%
Weight Uniformity	
Ph	6.4
Moisture Absorption	Good
Spreadability	Uniform
Strip Loading Uniformity	Consistent

Table 8 FTIR Spectrum Assignments with Corresponding ethanolic extract of formulation

Wavenumber (cm^{-1})	Functional Group	Assignment	Related Ingredients / Phytoconstituents
3316.96	O–H	Stretching	Turmeric, Green tea, Terminalia chebula (phenols)
2924.45	C–H	Stretching	Cinnamon, eucalyptus oil, coconut oil
1610.43	C=C	Aromatic stretching	Green tea, rose (flavonoids)
1513.54	Aromatic ring	Skeletal vibration	Polyphenols (rose, tea)
1444.41	C–H	Bending	Plant-derived hydrocarbons
1327.43	C–N	Stretching	Alkaloids (polyherbal components)
1205.72	C–O	Stretching	Phenols, ethers (turmeric, tea)
1154.86	C–O–C	Stretching	Essential oils
1015.45	C–O	Glycosidic linkage	Carbohydrates (plant materials)
862.84	C–H	Bending	Aromatic compounds
758.85	C–H	Bending	Substituted aromatics

Table 9 GCMS Peak Assignments with Corresponding ethanolic extract of formulation

Peak No.	**RT (min)***	**Compound**	**Major Plant Source**	**Biological Role**	**Relevance**
P1	~4.2	Anisole	Plant metabolite	Mild antimicrobial	Supportive
P2	~6.6	m-Cymen-8-ol	Eucalyptus	Antimicrobial, antioxidant	Skin protection
P3	~7.5	Thymol	Cinnamon / Eucalyptus	Strong antimicrobial, anti-inflammatory	Potent Anti-inflammatory action
P4	~10.4	Camphor	Eucalyptus	Anti-inflammatory, analgesic	Symptom relief

P5	~10.8	(+)-2-Bornanone	Eucalyptus	Anti-inflammatory	Skin soothing
P6	~13.5	Coumarin	Terminalia chebula	Anti-inflammatory, antioxidant	Potent Anti-inflammatory action
P7	~14.5	(Z)-3-Phenylacrylaldehyde	Cinnamon	Antimicrobial	Infection control
P8	~17.8	Spathulenol	Tridax / Eucalyptus	Anti-inflammatory, antimicrobial	Wound healing
P9	~18.9	γ-Himachalene	Essential oils	Anti-inflammatory	Skin disorders
P10	~19.7	Caryophyllene-type alcohol	Plant oils	Anti-inflammatory	Potent Anti-inflammatory action
P11	~20.5	Isobornyl acetate	Eucalyptus	Antimicrobial	Wound healing
P12	~21.0	Longipinocarveol	Eucalyptus	Anti-inflammatory	Skin repair
P13	~22.1	β-Nootkatol	Essential oils	Antimicrobial	Infection control
P14	~23.8	ar-Turmerone	Curcuma longa	Strong anti-inflammatory	CORE anti-psoriatic
P15	~24.1	Turmerone	Curcuma longa	Immunomodulatory	Potent Anti-inflammatory action
P16	~24.5	Curlone	Curcuma longa	Anti-inflammatory	Skin repair
P17	~25.0	(E)-Atlantone	Curcuma longa	Anti-inflammatory	Potent Anti-inflammatory action
P18	~25.4	(Z)-Atlantone	Curcuma longa	Anti-inflammatory	Potent Anti-inflammatory action
P19	~26.0	γ-Atlantone	Curcuma longa	Anti-inflammatory	Skin inflammation
P20	~26.5	α-Atlantone	Curcuma longa	Anti-inflammatory	Potent Anti-inflammatory action
P21	~27.5	Columbin	Tridax procumbens	Anti-inflammatory	Wound healing
P22	~28.5	Isobornyl thiocyanoacetate	Essential oils	Antimicrobial	Infection control
P23	~29.5	Cyclohexanediol derivative	Terpenoid	Anti-inflammatory	Skin healing
P24	~30.5	Methoxytropone	Plant metabolite	Antimicrobial	Skin infections

P25	~31.5	Benzylidenemalonaldehyde	Cinnamon	Antimicrobial	Infection control
P26	~32.5	2-Propenal, 3-phenyl	Cinnamon	Antimicrobial	Antibacterial
P27	~33.5	cis-2-Methoxycinnamic acid	Cinnamon	Antioxidant	Skin healing
P28	~34.5	o-Acetoxycinnamic acid	Cinnamon	Anti-inflammatory	Potent Anti-inflammatory action
P29	~35.5	Cinnamaldehyde dimethyl acetal	Cinnamon	Antimicrobial	Skin infections
P30	~36.5	Caffeine	Green tea	Antioxidant, anti-inflammatory	Skin repair
P31	~38.5	Hydroxycinnamic acid derivative	Terminalia / Tea	Antioxidant	Potent Anti-inflammatory action

Table 10. GCMS Peak Assignments with Corresponding ethanolic extract of formulation

FTIR Peak (cm^{-1})	**Bond Type**	**Functional Group**	**Representative Compounds**
3400–3200	O-H stretching	Alcohol / Phenol	Thymol, Coumarin, Hyroxycinnamic Acid
3000-2850	C-H stretching	Alkane	Tumerones, Camphor
1750–1700	C=O stretching	Carbonyl (Ketone/Aldehyde)	Camphor, Cinnamaldehyde
1680–1600	C=C stretching	Alkene	Cinnamic Derivatives
1600-1500	C=C bending	Aromatic ring	Coumarin, Caffeine
1450-1375	C-H bending	Alkane	Terpenoids
1300-1000	C-O stretching	Ether/Ester	Isobornyl Acetate
900-700	C-H bending	Aromatic substitution	Phenyl Derivatives

Table 11. Time-Kill study of Polyherbal formulation against Staphylococcus aureus

Time (min)	Log_{10} Reduction
0	0.0
1	1.1
5	3.2
10	4.5
15	4.8

Table 12. Integrated Comparative Analysis of Antimicrobial Activity and Synergy

Parameter	Polyherbal Formulation (A)	Herbal Mixture	Interpretation
MIC	Reduced	Increased	Enhanced potency in formulation
ΣFIC Index	≤ 0.5	~1.25	Synergistic effect vs additive interaction
Interaction Type	Synergistic	Additive	Strong vs weak interactions between phytoconstituents
Activity Level	Strong inhibition	Moderate inhibition	Increased antimicrobial performance