Transdermal Diffusion Studies of a Polyherbal Ointment and its tropical therapy

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SUMMARY: Transdermal diffusion studies of a polyherbal ointment were carried out in vitro using franz- Diffusion cell and microbial one inhibition diameter as drug release monitoring parameters. Out of the three ointment base prepared, (Hydrocarbon Base (HB), surfactant Base (SB), Surfactant Base + (Penetration Enhancer (10 % DMSO) SBPE), we observed that SBPE possesses maximum drug release capacity, as indicated by simultaneous high performance liquid chromatograph (HPLC) and microbial one inhibition monitoring studies. Clinical observations showed at SBPE was effective in cases like infected wounds, scratches and abrasions, infected scabies, pyoderma and furunculosis. The overall cure rate s 79.3% with the antimicro-bial ointment.

INTRODUCTION

Poly herbal formulations especially Ayurvedic products, are no evaluated in a proper manner in most of the quality control labs, the major draw back of the analysis, of these formulations is the lack of supply of the constituents having real pharmacological activity overlapping of spectal data etc, lack of availability of chemical markers and of bio availability test etc. As the present work we have made an attempt to analyses an anti-microbial ointment by utilizing high performance liquid chromatography & bacterial zone inhibition studies which is used as a biopredictor. The clinical efficiency of the ointment was received by choosing certain infections, skin diseases like, infected scabies, abressive scratches, pyoderma, etc.

MATERIALS & METHODS
(Raw Materials stattardisation)

All the raw material used for the preparation of different ointment bases were standardized as per the I.P and B.P standards.

All chemicals and organic solvents used were of analytical grade. Crude drugs selected for this study were authenticated at the Tamil nadu Agricultural University, coimatore and also at the department of Pharmacognosy and photochemistry, SRIPMS, Coimbatore. Various Pharmacognostic, phytocemical , physical and microbial contamination tests were carried out according to the standard procedures as described in I.P., British herbal pharmacopoeia, Ayurvedic pharmacopoeia Ayurvedic Pharmacopoeia of India, W.H.O guidelines and also by referring to various published literatures.1,2,3,4,5,6,7,19.

Five crude drugs reported to have a good antimicrobial activity.8,9,10. Were selected for making the ointment formulation. They were Nigella sativa, Rubia cordifolia,....
Pongamia glabra, Mallotus-phillipinesis and psorelea corlifolia. Suitable solvent extracts were prepared from these crude drugs according to the positive tests indicated by phytochemical analysis (Table-1).

**Table 1 Solvent**

Pretocol for solvent extraction of crude drugs

| Sl. No | Drugs used                  | Official part                  | Wt of saper | Vol of org. Solution | Duration of extract | Yield     |
|--------|-----------------------------|-------------------------------|-------------|----------------------|---------------------|-----------|
| 1      | Nigella sativa              | Seeds                         | 500g        | 70% ethanol          | 1 week              | 24.8 % W/W|
| 2      | Rubia cardifolic            | Stem                          | 500g        | 70% ethanol          | 2 week              | 19.2 % W/W|
| 3      | Pongamia glabra             | Seeds                         | 250g        | Per ether for fatty and methanol for extract | 2hrs               | 37.6 % W/W|
| 4      | Mallotus phillipinesis      | Glands and hairs of fruit seeds | 200g        | Absolute alcohol     | 3hrs               | 3.2 a%    |
| 5      | Psorelea Conlifolia         |                               | 500g        | Per ether for defaulty methanol | 5hrs               | 20.4 %    |

**APPARATUS AND CONDITIONS**

HPLC Marking procedure for Individuals Drugs water HPLC system was used for the analysis. The column used was Zorbax C8 Reverser phase column). A mixture of acetonitrille: 50ml disodium hydrogen orthophosphate (adjusted to pH3.5 using orthophosphoric acid) 50:50 v/v was used as moil phase at a flow rate of 1.5ml/minute with an operating pressure of 3000psi. A rheodyne 7125 injection of samples. Detection was done t 215nm, with a sensitivity of 0.2 AUFS. The mobile phase was filtered trough 0.45u membranes filter and degassed. The separation was carried out at the room temperature of about 20°C (11, 12).

**PREPARATION OF STANDARD SOLUTIONS**

Standard stock solutions of P.glabra (PG), Psorelea corylifolia (PC), N. Sativa (NS), R. corlifolia (RC) and M.Phillipinesis (MP) were prepared after suitable dilution wit mobile phase, 20uL of standard stock solution of PG, PC, NS, MP, RC were injected and the chromatograms were recorded. From the individual chromatogram the peaks of individual drugs were observed and the peaks for analysis were selected which are not interfering with the peaks of other drugs in the chromatogram. Again another chromatogram was recorded by combining all drugs extracts n equal quantities (4uls each) to make a volume of 20u ls. A computer controlled data station with baseline 810 software was used to plot the peak area Vs. Concentration in µg/ml.

**PREPARATION OF OINTMENT BASES**
Three ointment bases were prepared for the evaluation. They were (a) hydrocarbon base (B), (b) surfactant base (SB), (c) surfactant base with a penetration enhancer (10% dimethyl sulfoxide) designated as SBPE. All the ointment bases were formulated using chemicals of a analytical grade and prepared as per B.P. standards. Plant drug extracts were incorporated individually in all the four bases. As there are five different types included in the ointment bases, 10% of each drug was added to the ointment bases and homogenized.

**TRANSDERMAL ABSORPTION STUDIES**

Diffusion cell 10cm x 2.3cm dimension was used for the study. 2.5gms of the abdominal rat skin, which was tied to the diffusion cell in a way that the ointment occupies the inner side of the diffusion cell. The diffusion cell was placed in a beaker (250ml capacity), containing 100ml of pH 7.2 buffer. In order to maintain uniform mixing the medium was stirred at a constant speed with a magnetic stirrer. At regular intervals of time (every one hour up to 5th hour); withdrawn specific volume of the medium (2ml) form the diffusion cell and maintained the level of the medium constant by replacing fresh medium into the cell. The whole diffusion cell was placed in an organ broth at 37°C ± 0.5°C. These withdrawn samples were diluted (100times) with the mobile phase of the HPLC system and analyzes 14-16. simultaneously these withdrawn samples at definite time intervals were tested for its antimicrobial activity by zone imbibitions diameter method using two different kinds of bacterial strains (gram positive and gram negative), and two strains of fungi (candida albicans, Cadida tropicalis). The experiment was repeated three times for individual ointment bases and tabulated the mean of the values. From HPLC data the individual drug release in the withdrawn samples were identified. (Table 2-7).

| Table -2 |
| Drug release concentration profiles for different ointment formulations from HPLC data (HB, SB, SBP) |
| Drug release profiles in mg/grams of total drug extract |

| Formulation | 1hr | 2hr | 3hr | 4hr | 5hr | Total Drug Released at the end of 5th hour |
|-------------|-----|-----|-----|-----|-----|------------------------------------------|
| SBP         | 0.3221 | 0.71438 | 0.7628 | 0.7686 | 0.7733 | 3.5448 |
| HB          | 0.1090 | 0.1461 | 0.1752 | 0.2135 | 0.2260 | 0.8698 |
| SB          | 0.62504 | 0.7299 | 0.8009 | 0.8586 | 0.9035 | 3.0139 |

| Table -3 |
| Chemical marking procedures for individual drugs using high performance Liquid chromatography |

| Drug                  | Retention time | Specific peak selected as marker | Peak Area | Peak Height | Solution concentration (ngs/ml) |
|-----------------------|----------------|---------------------------------|-----------|-------------|---------------------------------|
| Pongamia glabra       | 1.40 2.29 3.467 | 3.467                           | 266209    | 16316       | 6.82                            |
| Drug             | Time in Hours | Zone pf Inhibition in diameter (mm) |
|------------------|---------------|------------------------------------|
|                  | 1hr           | SBPE  | SB    | HB     |
| Psorelea conlifolia | 10.26 ± 0.288 | 8.01 ± 0.312 | 8.10 ± 0.278 |
|                  | 2hr           | 18.51 ± 0.272 | 18.22 ± 0.276 | 0.32 ± 0.266 |
|                  | 3hr           | 18.42 ± 0.229 | 16.76 ± 0.277 | 8.66 ± 0.298 |
|                  | 4hr           | 18.42 ± 0.284 | 16.70 ± 0.398 | 9.28 ± 0.394 |
|                  | 5hr           | 18.50 ± 0.347 | 16.72 ± 0.292 | 9.32 ± 0.321 |

**Table -4**

Drug release profile for different ointment bases monitored by microbial zone of inhibition diameter

**Micro Organism used:** Escherichia coil (gram-ve)
### Table – 5
**Micro Organism used: Staphylococcus aureus (gram-ve)**

| Time in Hours | Zone of Inhibition in diameter (mm) | SBPE | SB | HB |
|---------------|------------------------------------|------|----|----|
| 1hr           |                                    | 5.16 ± 0.477 | 9.11 ± 0.288 | 6.07 ± 0.342 |
| 2hr           |                                    | 16.92 ± 0.277 | 14.32 ± 0.276 | 6.12 ± 0.387 |
| 3hr           |                                    | 18.08 ± 0.216 | 14.5 ± 0.312 | 7.20 ± 0.261 |
| 4hr           |                                    | 18.04 ± 0.321 | 14.62 ± 0.264 | 7.23 ± 0.293 |
| 5hr           |                                    | 18.03 ± 0.321 | 15.0 ± 0.292 | 7.31 ± 0.217 |

SB – Surfactant base  
SBP – Surfactant base = Permeation enhancer  
HB – Hydrocarbon base  
(100% DMOO)

### Table – 6
**Micro Organism used: Candida albicans**

| Time in Hours | Zone of Inhibition in diameter (mm) | SBPE | SB | HB |
|---------------|------------------------------------|------|----|----|
| 1hr           |                                    | 16.92 ± 0.229 | 8.92 ± 0.424 | 5.5 ± 0.424 |
| 2hr           |                                    | 20.32 ± 0.286 | 12.02 ± 0.398 | 5.92 ± 0.312 |
| 3hr           |                                    | 20.48 ± 0.394 | 12.24 ± 0.226 | 6.28 ± 0.298 |
| 4hr           |                                    | 21.0 ± 0.314 | 12.36 ± 0.310 | 8.42 ± 0.29 |
| 5hr           |                                    | 20.64 ± 0.298 | 13.31 ± 0.348 | 8.5 ± 0.346 |

### Table – 7
**Micro Organism used: Candida Tropicalis**

| Time in Hours | Zone of Inhibition in diameter (mm) | SBPE | SB | HB |
|---------------|------------------------------------|------|----|----|
| 1hr           |                                    | 12.8 ± 0.342 | 14.2 ± 0.342 | 5.0 ± 0.232 |
| 2hr           |                                    | 20.48 ± 0.286 | 19.60 ± 0.198 | 5.18 ± 0.286 |
| 3hr           |                                    | 21.36 ± 0.512 | 20.45 ± 0.414 | 5.36 ± 0.394 |
| 4hr           |                                    | 22.92 ± 0.492 | 20.76 ± 0.472 | 6.26 ± 0.261 |
| 5hr           |                                    | 22.68 ± 0.311 | 20.95 ± 0.268 | 6.33 ± 0.386 |

SB – Surfactant base  
SBP – Surfactant base = Permeation enhancer  
HB – Hydrocarbon base  
(100% DMOO)

**PREPARATION OF THE SKIN MEMBRANE**
The excised shaven abdominal skin [3.5, 3.5] of five to seven weeks old albino mouse of either sex was used. The skin was washed with distilled water placed in normal saline for 5-10 minutes and used for fixation in the diffusion cell.\textsuperscript{17}

**TOPICAL SENSITIVITY TEST**

All the four ointment (AB, HB, SB and SBPE) were tested for their skin sensitivity tests by applying to the elbow of the hand in selected human volunteers and observed the side effects if any, as a set of parameters like skin inflammation, skin irritation reddening of skin (allergic reactions) etc. None of the four ointments showed any allergic reactions.

**CHEMICAL MAKING PROCEDURES**

The characteristic peak responsible for the crude was selected and kept ad the marker for the particular drug. In a similar manner all drugs were characterized by their specific peaks which were not overlapping with one another.\textsuperscript{18} The characteristic peaks responsible for individual drugs were tabulated as shown in (Table -2).

**CLINICAL TRIAL METHOD**

30 patients of the dermatology Out – patient Department, chaitanya Nursing Home North parur, Ernakulam, Kerala, were selected for study, including various primary and secondary infections like abrasions and scratches, infected wounds infected scabies pyoderma and furunculosis.

A pre-treatment culture swab was initially taken from each patients skin lesions to examine sensitivity. The patients were subsequently instructed to apply herbal antimicrobial ointment dispensed to them the herbal ointment was to be applied insufficient quantity over the skin lesions twice a day after initial removal of the crusted exudates with saline soaks.

The clinical diagnosis and anatomical distribution were recorded in every patient. Six patients in each of the cases mentioned, were selected for experimenting the efficacy of ointment, the maximum duration allotted was for a period of 7 days for monitoring % curation rate. The criteria for clinical assessment are summarized in Table – 8, 9 and 10.

| Cure Rate       | Complete Clearance of Lessions                                      |
|-----------------|---------------------------------------------------------------------|
| 25% improvement | +( Very little noticeable improvement )                              |
| 50% improvement | ++(Discharge), Scab ++, Moderate Erythema                           |
| 75% improvement | ++++ no visible discharge, pyogenic scab has cleared & very little Erythema |
| 100% improvement| +++++ Completely cured.                                            |
### Table -9
Curative potency of the formulation SBPE

| Type of lessons                        | Days cure Rate % |          |          |          |          |          |          |
|----------------------------------------|------------------|----------|----------|----------|----------|----------|----------|
| Abrasions and scratches Case - I       |                  | 1        | 2        | 3        | 4        | 5        | 6        | 7        |
|                                        | Nil              | 4.16     | 8.3      | 29.16    | 33.3     | 75.0     | 83.8     |
| Infected wounds Case - II              |                  | 8.3      | 12.5     | 20.8     | 29.16    | 50.0     | 79.16    |
| Infected scabies Case – III            |                  | 4.20     | 8.3      | 25.0     | 37.5     | 50.0     | 79.2     |
| Pyoderma Case – IV                     |                  | 5.20     | 12.5     | 20.5     | 37.5     | 70.8     | 80.5     |
| Furunculosis Case - V                  |                  | Nil      | Nil      | 8.3      | 16.6     | 29.16    | 58.33    | 75.0     |

### Table – 10
Curative potency of the formulation studies (SBPE)

| Cases        | Cured | Improved | Total |
|--------------|-------|----------|-------|
| Case - I     | 2     | 4        | 6     |
| Case – II    | 1     | 4        | 6     |
| Case – III   | 1     | 4        | 6     |
| Case – IV    | 1     | 3        | 6     |
| Case – V     | -     | 4        | 6     |
| Total        | 5     | 19       | 30    |
| %            | 16.0  | 63.33    | 79.33 |

SBPE: Surfactant Baset Penetration enhancer

As mentioned in Table 10 30 patients were selected in this screening studies of which 5(16%) were cured and 19 were improved (63.33%) which included 5 different cases mentioned. The overall clinical success rate was 79.33% with this polyherbal antimicrobial ointment.

**RESULTS AND DISCUSSIONS**

The percutaneous absorption studies monitored by the bacterial one inhibition method to predict the efficacy of an ointment formulation to release drugs can be very well used as a biopredictor for this type of drug trials.

For a poly herbal ointment it is very difficult to study the drug release profiles as well as the concentration of the drug released at every oral. Monitoring with HPLC for finding the release pattern for any type of ointment bases also provides important information regarding the capacity of the
ointment of release various active constituents. Also one can use HPLC for making different category of drugs according to its content of active constituents, this will be helpful again to monitor any particular drug in poly herbal ointment for its identification and also for finding out the concentration of the chemical substances released from base. Therefore the efficiency of the polyherbal ointment can be predicted confidently by utilizing these two techniques together i.e. the bacterial zone inhibition diameter (can be used as drug release monitoring parameter) and the HPLC monitoring (can be used as chemical marker) applying simultaneously to study sample drug solution at periodic time intervals from diffusion cell.

Topical sensitivity test for the ointments prepared, revealed the safety of the preparation i.e. without the production of any type of side effects. The data obtained from bacterial one inhibition diameter & HPLC showed that SBP (surfactant base + permeator enhance 10% DMSO) Having drug release maximum at the time interval of 2nd 3rd hour. Surfactant base (SB) is having a release maximum at 3rd hour to 4th hours. Hydro carbon base “drug release data” showed that it can release maximum at 3rd hour to 4th hour. Hydro carbon base “drug release data” showed that it can release drugs at a constant rate but the concentration of the drug release will be very less.

Clinical observation studies for the SBPE (surface base + Penetration Enhancer) showed that this, herbal antimicrobial ointment can be use days an effective topical medications for various infected skin conditions. The overall clinical success rate was 79.33% with this polyherbal ointment preparation.

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