PHYSICOCHEMICAL PROPERTIES: A TOOL TO PREDICT CLINICAL EFFICACY OF MUDS

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ABSTRACT

Muds differ in their constituents depending on their geographical and topographical origin. As mud is a mixture of many different types of organic and inorganic complex substances and isolating any one out of them is very difficult, the present study was aimed towards efforts to correlate some of the physicochemical properties like colour, pH, electrical conductivity, elemental composition and humic acid content of different types of muds with their therapeutic efficacy in the treatment of inflammatory skin disorder like psoriasis. Muds from four different regions of India were collected and it was observed that mud which was collected from Dwarka (Gujarat, India) was light yellow in colour, neutral in pH, contained more of carbonates and was less effective in psoriasis. Amongst the two collected from Kerala (India) one was black in colour and the other was brown in colour. Black mud was slightly acidic, rich in elements (Ca, Mg) and humic acid and had highest electrical conductivity while brown was neutral, had optimum concentration of calcium and magnesium for skin penetration and was more effective on psoriasis. The one collected from Vadodara (India) was reddish brown in colour, neutral in pH, and was found effective secondary to Kerala Brown, in psoriasis. Correlating the data of clinical studies with soil’s physicochemical properties like colour, pH, electrical conductivity, humic acid content, and concentration of elements of soil we could conclude that the soil which was, brown or reddish brown in colour, neutral in pH, and which had calcium and magnesium and humic acid concentration in the range as to effect therapeutic skin penetration, would be used effectively against inflammatory proliferative disease like psoriasis.
KEYWORDS: muds; elements; skin penetration; humic acid; physicochemical properties; psoriasis.

INTRODUCTION

Mud (wet soil) has been associated with human beings right from the origin of life. It is said in Indian philosophy and literature (Ayurved) that man is made of mud and disintegrates into it after death. Only skeleton is found as remnants of buried persons: flesh, skin, organs all disintegrate into mud. Moreover, mud also has emotional attachment with human beings. Each individual experiences a special emotional attraction to the place where he lives. This would probably be due to geomagnetic attraction between the self and the place from which that self has been composed (i.e. from the food grown in that soil). Ayurved, an Indian medicinal scripture describes importance of relationship of soil and human beings. It also describes relations between rainfall of a region, with therapeutic uses of that soil, therapeutic uses of vegetation grown on that soil and the diseases prevalent in human beings residing in that region.

Mud contains many complex substances, out of them elements like Calcium, Magnesium, Sodium, Potassium and Phosphorus play a vital role in skin integrity. Excess or less amount of these elements in skin cause deviation from the normal path of cell differentiation and proliferation of skin cells, leading to generation of proliferative skin diseases like psoriasis as explained in the following text.

Psoriasis is a condition of unregulated cell proliferation. Magnesium and calcium are regarded as strictly intracellular cations. Calcium plays a major role in regulating epidermal functions, including cell proliferation, terminal differentiation, and cell-to-cell adhesion. Aberrations in calcium regulation have been noted in psoriasis when levels of the calcium binding protein calmodulin are elevated and the normal calcium gradient within the epidermis is altered. It is known that part of the intracellular calcium is bound to protein and is thought to be an integral part of the cell periphery or cell membrane. It is established that in psoriatic patients, calcium binding protein calmodulin, calgranulins A&B, S100 proteins A8 & A9 are in large amounts and there is altered calcium metabolism. Ca+2 has also been shown to play an important role in apoptosis (Programmed cell death), which is currently a hot subject for the obvious reason that the final differentiation step between the stratum granulosum level and the stratum corneum represents a particular aspect of programmed cell death.
Sodium is the main extracellular cation and like the chloride concentration, the concentration of sodium is unusually high in the skin as compared with other organs. It has been recognized that the sweat (extracellular area) excreted from those affected with psoriasis contains twice the amount of sodium and four times the amount of potassium in comparison to those of normal individual.\[9\] Salivary sodium levels were also significantly elevated in psoriasis.\[10\] Scanning electron-microscopy in combination with Secondary Electron Imaging and X-ray Energy Spectrometry (Electron Probe Micro Analysis) on the stratum corneum of psoriatic skin indicated significant elemental alterations including an increase in phosphorus and calcium levels.\[11\] In a 31P Magnetic resonance spectra of psoriatic skin, it was observed that there was elevations in phosphomonoester concentrations and phosphomonoester/phosphodiester ratio and in conjunction with chromatographic analysis, Heng MC\[12\] also mentioned that there was a defect in phosphometabolism in psoriatic skin.\[13\] Vitamin D analogs have found to be very useful in treating psoriasis as it is established that Vit. D controls Calcium and Phosphorus metabolism and itching.\[14\] In a research conducted to study the effect of elements on tissue culture of psoriatic skin and normal skin, the number of cells and their cyclic AMP content were used as parameters for cell division and for proving the selective involvement of magnesium salts in the antiproliferative effect. The results showed that the inhibitory effects of magnesium bromide and magnesium chloride on cell growth were significantly stronger than those of their corresponding potassium salts or of sodium salts of Dead Sea water on psoriatic cells.\[15\] Elemental distribution of potassium in psoriatic skin varies with the functional state of the keratinocytes, e.g., electrolytes influence cell metabolism and cell proliferation, and trace elements play a crucial role in a great number of enzymes. As high K levels prevent the Ca-induced differentiation of keratinocytes, high K levels may be the cause of the high cell differentiation in psoriatic skin.\[16\] In chronic lesions of psoriasis, if all layers of the lesion were analyzed in total, the potassium content was found to be greatly increased from an average of 259.5 mg to an average of 491.5 mg in 100 gm. dry tissue.\[17\] This change might be caused simply by the acanthosis: the cellular content of psoriasis lesions is much greater than normal, and epithelial cells are rich in potassium. The main cations inside the cell are potassium and magnesium. The relative amount of potassium is much higher, possibly because of the high potassium content of the epidermis and other cellular elements of the skin, such as follicular epithelium, sweat gland cells, sebaceous gland cells, endothelial cells, and fibroblasts. Soil humus refers to the amorphous organic materials remaining in soils after extraction of the water soluble fraction and exclusion of particulate organic materials. Humus consists of substances like humic acids, fulvic acids, humins,
hymatomelanic acid and products of advanced decomposition of organic residues and products resynthesized by micro-organisms (protein like substances, carbohydrates, waxes, fats, tannins, lignins etc.[18] Humic acids are usually regarded as polymers of aromatic compounds, and there is little doubt that aromatic structures are incorporated in the complex. They have been demonstrated by spectroscopy [19] by the detection of phenolic groups in functional-group analyses, by the release of simple aromatic compounds in various degradation procedures, and by the fact that artificial humic acids with many of the properties of natural humic acids can be prepared in the oxidative polymerization of simple phenols.[20]

It is observed that psoriatic lesions contain less extracellular calcium, a condition which favors proliferation[21] and interaction of Humic Acid with epidermal cells directly increase their Ca$^{2+}$ permeability, thus over stimulating the Ca2+ second messenger system through activation of protein kinase and/or other Ca2+-dependent enzymes, thereby regulating proliferation and differentiation of keratinocytes.[22] Thus elements like Ca, Mg, Na, K, P and humic acid play an important role in skin integrity of psoriatic skin. To summarize, Na, K and P are found excess in psoriatic extracellular concentration and Ca and Mg being mainly intracellular ions, and responsible for regulating cell proliferation, their concentrations is found less in psoriatic cells. Moreover influence of Dead Sea water on magnesium level of cells, and that of humic acid on Ca$^{+2}$ uptake by increasing cell’s permeability both indirectly signify their role in treating human psoriatic lesions. Hence we aimed to study the penetration of these elements of mud into human excised skin and also to find out whether concentration of these elements in soil had any influence on their skin penetration. We also wanted to study that apart from the concentration of elements and humic acid, whether some of the soil’s physicochemical properties like colour, pH, and electrical conductivity, had any role to play to predict therapeutic efficacy of muds. For this purpose we tried to correlate the data of above mentioned physicochemical properties with clinical and ex-vivo studies.

**MATERIALS AND METHODS**

**Materials**

Samples of soil were collected from 4 different geographical regions of India namely (1) Kerala Black, Kerala (2) Kerala Brown, Kerala (3) Vadodara, Gujarat (4) Dwarka, Gujarat. Out of these 1, 2, and 4 soils had traditional therapeutic importance while Vadodara region was selected to see the effects of soil of the place we inhabit. Soil samples collected from Gopi Talav (a place of historic importance) Dwarka, Gujarat had less rainfall, Vadodara,
Gujarat (a place of our habitat) had moderate rainfall, Allepazhua, Kerala, had high rainfall and the land was irrigated by backwater lagoons (black colour, Kerala Black) and another sample from Allepazhua where the land was irrigated by river water (brown colour, Kerala Brown).

Soil was dug from a non-agricultural land, 3 to 4 ft. deep from ground level to assure freedom from litter, gravels, pesticides and pollutants. It was dried in sun for 10-12 days, milled, sifted through 40# sieve and stored in airtight container. Soil collected from Dwarka,(Gujarat) is termed as Dwarka, that from Vadodara(Gujarat) as Vadodara, that from Kerala which was black in colour as Kerala Black and that from Kerala which was brown in colour as Kerala Brown in the subsequent sections.

Freshly excised human abdominal skin was obtained from a Cosmetic Surgical hospital in Vadodara, India. Humic acid was obtained from Hi Media Laboratories Pvt. Ltd., Mumbai.

**METHODS**

**Colour**

Colour of the soil was determined on the basis of Munsell colour chart adopted by USDA (United States Department of Agriculture) as the official color system for soil research in the 1930s.

**pH**

pH of soil was determined by preparing 1:2 soil: water suspension, keeping it aside with frequent shaking for 1 hour, then filtering it through Whatman no.40 filter paper and measuring the pH of filtrate by pH meter (Elico Pvt. Ltd).[23]

**Electrical Conductivity**

Electrical conductivity was used as a measure of soluble salts concentrations present in soil.[24] Soluble salts present in soil dissociate into their respective cations and anions when dissolved in water and impart conductivity. Suspension of soil in the ratio 1:2(soil: water) was prepared in distilled water, kept aside for half hour with intermittent shaking and then centrifuged at 2000rpm for 5 min.[24] Supernatant was used to measure conductivity by conductivity meter (Elico CM 180).
Elemental Composition
Elemental composition was determined by ICP-AES (Inductively Coupled Argon Plasma Atomic Emission spectrometry). 200mg of soil (120um size) was accurately weighed, 20ml of distilled water was added to it, intermittently shaken and kept aside for 1 hr. It was then centrifuged at 2000rpm for 5 min and the supernatant was taken for analysis and concentration of Ca, Mg, Na, K, and P was measured (iCAP 6300 model).

Isolation of Humic acid from soil samples[25]
200 mg of each soil (120#) was taken in a 100 ml volumetric flask and to it 20ml of 0.5 M NaOH was added, shaken on a mechanical shaker for 1 hr. and kept overnight. Next day it was centrifuged and the liquid decanted into another beaker. To this, conc. HCl was added to achieve pH of 1-1.5. Humic acid was precipitated at the bottom of the beaker. The upper liquid was decanted and the precipitate (humic acid) was air dried and weighed. It was then dissolved in 20 ml of distilled water and the sample’s absorbance noted under UV1601 spectrophotometer (Shimadzu) at 224 nm. Pure Humic acid was supplied by Hi Media Laboratories Pvt Ltd., Mumbai. Solutions of 5, 10, 20, 30, 40, 50 ug/ml of humic acid were prepared in distilled water and calibration curve plotted by measuring absorbance at 224nm.

Ex-vivo skin diffusion study
Ex-vivo skin penetration studies were conducted on freshly excised healthy human abdominal skin obtained from a cosmetic surgery hospital. The skin was made free from fat bodies by cutting with scissors and stored in saline solution at -60°C. The study was completed within 3 days of receipt of the skin. For the study, the skin was kept in distilled water, allowed it to equilibrate, washed clean and cut into pieces of approximately 3x3 cms². Diffusion studies were carried out in Keshrey –Chein diffusion cell having internal diameter 2.0 cms. The receptor compartment was filled with distilled water (20ml) and the donor compartment contained 200mg of soil (120 um size) wetted by 1ml distilled water. Each skin piece, with its stratum corneum side exposed to the air (donor compartment), was mounted horizontally across the upper chamber of the diffusion cell. The two chambers were held tightly by four springs to prevent any leakage of donor material. The temperature of the receptor fluid was maintained at 37°C. and the fluid was stirred by Teflon coated magnetic bar at low speed (60 rpm). The experiment was conducted for 60 minutes and thereafter the receptor fluid was filtered by Whatman filter paper and stored in refrigerator until analyzed by ICP-AES for 5 major elements i.e. Ca, Mg, K, Na, P and humic acid by Ultraviolet
spectroscopy. The skin was then removed from the cell, washed with distilled water, wiped dry and weighed accurately. It was then homogenized in a tissue homogenizer with 10 ml of distilled water three times. This extract was then centrifuged, filtered through Whatman filter paper no.40 and stored in refrigerator until analyzed by ICP-AES for Ca, Mg, K, Na, P and humic acid by Ultraviolet spectroscopy. The whole set of experiment was conducted in triplicate for each of the 4 muds. Blank experiment was run in triplicate in the same manner without the soil to measure the elemental and humic acid concentration of skin and receptor medium.

Preliminary clinical study

Preliminary clinical studies were conducted to study the efficacy and safety of mud. This study was conducted at Dermatology Department of Sir Sayajirao General (SSG) Hospital, Vadodara, India and at Government Ayurved Hospital, Vadodara, India. At both the hospitals, the study was conducted under the supervision of a Dermatologist and patients were monitored and evaluated by them. In the case of psoriasis, PASI (Psoriasis Area Severity Index) score was noted, patients observed for 3 months and were evaluated every 15 days. Patients’ feelings of well-being were also noted.

A protocol for the study was prepared and Informed Consent Form was filled up by the participants. A Case Report was also prepared wherein the fortnightly observations and symptomatic results of patients were recorded. Photographs of the affected part were also taken during periodical examination. In all 20 patients of both sex (5 for each mud) having psoriasis, in the age group of 18 to 60 years participated in the study. The patients were asked to wet the soil, apply on the affected part and leave it on the skin for 30 minutes. After 30 min., mud was gently scrapped off the application site.

RESULTS AND DISCUSSIONS

The observations of color, pH and electrical conductivity, elemental composition, humic acid concentration and ex-vivo skin penetration of elements and humic acid are tabulated in table no.1.
<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Characteristics</th>
<th>Kerala Black</th>
<th>Kerala Brown</th>
<th>Dwarka</th>
<th>Vadodara</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Color, Munsell notation</td>
<td>Hue N Value 1.7 Black</td>
<td>Hue 5YR Value 2 Brownish Black</td>
<td>Hue 5Y Value 8 Chroma 2</td>
<td>Hue 5YR Value 3 Chroma 2</td>
</tr>
<tr>
<td>02</td>
<td>pH</td>
<td>5.89±0.02</td>
<td>7.79±0.01</td>
<td>7.14±0.01</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>03</td>
<td>Electrical conductivity in mS</td>
<td>9.5±0.04</td>
<td>4.97±0.09</td>
<td>0.95±0.02</td>
<td>1.51±0.08</td>
</tr>
<tr>
<td>04</td>
<td>Ca content (µg/100mg soil)</td>
<td>27.88±0.02</td>
<td>4.81±0.01</td>
<td>10.59±3.06</td>
<td>6.94±0.03</td>
</tr>
<tr>
<td>05</td>
<td>Mg content (µg/100mg soil)</td>
<td>45.61±5.78</td>
<td>10.37±0.04</td>
<td>5.94±0.04</td>
<td>2.79±0.03</td>
</tr>
<tr>
<td>06</td>
<td>Na content (µg/100mg soil)</td>
<td>38.64±4.01</td>
<td>93.5±10.06</td>
<td>36.73±6.04</td>
<td>33.92±4.04</td>
</tr>
<tr>
<td>07</td>
<td>K content (µg/100mg soil)</td>
<td>3.16±0.04</td>
<td>10.5±3.04</td>
<td>5.71±0.01</td>
<td>2.16±0.02</td>
</tr>
<tr>
<td>08</td>
<td>P content (µg/100mg soil)</td>
<td>0.26±0.01</td>
<td>0.16±0.01</td>
<td>0.33±0.01</td>
<td>0.50±0.01</td>
</tr>
<tr>
<td>09</td>
<td>Humic acid content %</td>
<td>0.182±0.014</td>
<td>0.122±0.022</td>
<td>0.086±0.006</td>
<td>0.076±0.008</td>
</tr>
<tr>
<td>10</td>
<td>Fe content in %</td>
<td>7.72</td>
<td>9.51</td>
<td>3.49</td>
<td>7.12</td>
</tr>
<tr>
<td>11</td>
<td>Skin penetration of Ca in %</td>
<td>23.09±2.06</td>
<td>45.53±5.08</td>
<td>30.78±4.09</td>
<td>44.66±6.07</td>
</tr>
<tr>
<td>12</td>
<td>Skin penetration of Mg in %</td>
<td>10.89±1.04</td>
<td>83.22±8.07</td>
<td>40.06±4.06</td>
<td>40.86±5.06</td>
</tr>
<tr>
<td>13</td>
<td>Skin penetration of Na in %</td>
<td>92.13±9.09</td>
<td>40.98±4.05</td>
<td>61.2±6.06</td>
<td>38.26±3.05</td>
</tr>
<tr>
<td>14</td>
<td>Skin penetration of K in %</td>
<td>81.32±4.08</td>
<td>9.71±0.04</td>
<td>10.85±1.05</td>
<td>27.77±4.07</td>
</tr>
<tr>
<td>15</td>
<td>Skin penetration of P in %</td>
<td>84.61±6.08</td>
<td>73.61±8.06</td>
<td>45.18±5.08</td>
<td>24.75±3.06</td>
</tr>
<tr>
<td>16</td>
<td>Skin penetration of Humic acid %</td>
<td>1.899±0.01</td>
<td>1.636±0.01</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

**Colour**

Soil colour is the property which exerts least influence on the other soil properties, but is important because it is largely a synthesis and expression of all other soil properties. Clearly, the observation of a given soil colour can be useful in the description, mapping, and classification of soils and may elucidate problems associated with soil genesis or soil evaluation.\[26\], \[27\] Soil colour has also been used to quantify soil organic carbon and iron contents.\[28\]

Soil colour depends on the types of soil constituents\[29\] and it is also easily recognizable and relatively stable over time, which explains why it is used for soil identification and qualitative determinations of soil characteristics. Iron is usually the most important mineral colouring
agent in soils, especially in clays. In ferruginous soils, the two common forms of free iron oxide produce red to yellow-brown colours (Kerala Brown and Vadodara). Calcium carbonate can also be significant when present in large amounts and may contribute to whitish-grey or yellowish-white colour (Dwarka). Organic matter may influence soil’s colour to an over-riding degree. The more humified the organic matter, the darker the colour as humins and humic acids contrast with the lighter coloured fulvic acids (Kerala Black). Thus colour examination predicted that Kerala Black (lagoon water irrigation) would be rich in humus, Kerala Brown (river water irrigation) would have mixture of humus and iron, Vadodara (Medium rainfall) would be rich in iron and Dwarka (scanty rainfall) would be rich in calcium carbonate. These predictions were found true on chemical examination as shown in table no.1. and in other results not disclosed here. Hence colour of soil can be used as a property to know iron, humic acid, and calcium carbonate content of soil. As humic acid and calcium play role in skin integrity in psoriasis, a soil having brownish or yellowish-white colour has a probability of being used in treatment of human psoriasis.

**pH**

pH of Kerala Black was acidic while that of Kerala Brown, Vadodara, and Dwarka was found to be near to neutral as shown in table no.1. In soil water system some of the adsorbed hydrogen ions dissociate from the surface of the soil colloids in to the soil solution. These dissociated H⁺ ions give rise to active or soluble acidity, but there are many more hydrogen ions still adsorbed on the soil colloids which give rise to potential acidity of the soil. Thus effective hydrogen ion concentration includes hydrogen from all sources such as those arising by dissociation of soluble acids and those dissociated from soil colloids. Low acidity of Black soil may be attributed to high concentration of calcium compared to other soils (refer table no.1) because sodium ions are held less strongly than the micelle calcium ions and are therefore more easily hydrolyzed than calcium ions and hence the pH of sodium saturated soils is higher than that of calcium saturated soils.[26] Clinical observations suggested that patients who were applied Kerala Black soil experienced irritation (due to acidity) while those who were applied other three soils showed no irritation. Thus pH of the soil can be used to predict whether it would be comfortable to apply on the psoriatic skin.

**Electrical Conductivity (EC)**

Although electrical conductivity does not provide a direct measurement of specific ions or salt compounds, it has been correlated to concentrations of nitrates, potassium, sodium,
chloride, sulfate, and ammonia (USDA NRCS guidelines for soil EC). Because salts move with water, low areas, depressions or other wet areas where water accumulates, tend to be higher in electrical conductivity than surrounding higher-lying, better drained areas. Soil microorganism activity declines as electrical conductivity increases. Electrical conductivity is a measure of soluble salts which can dissociate into ions.\textsuperscript{24}

Electrical conductivity was higher of Kerala Black than other soils. This may be attributed to high salt concentration and the fact that it was collected from land constantly water logged with back water lagoons. Kerala Brown was collected from river water irrigated land and so its EC was less than Kerala Black. Dwarka was collected from scanty rainfall region and Vadodara from moderate rainfall region so their EC is comparatively less. Collection location was reflected in ECs of these soils. Thus EC can be used to predict soil’s salt concentrations, origin and microbial activity.\textsuperscript{26} However, high salt concentration does not necessarily mean more penetration through skin, which is evident from following ex-vivo studies but it can surely indicate less antimicrobial activity of soil, which is evident in another study conducted by us (un-published data).

**Ex-vivo studies**

Skin remained in hydrated condition due to presence of water in receptor compartment and donor compartment (1ml). The hydrated stratum corneum has an affinity for both water-soluble and lipid-soluble nonelectrolytes.\textsuperscript{30} The major pathway for the skin permeation of water–soluble molecules is primarily transfollicular, i.e., through cells and cell membranes alike without discrimination. On the other hand, the pathway for lipid-soluble molecules is not well understood and presumably follows the endogenous lipids within the stratum corneum. These lipids are located both intracellularly and between the keratin filaments within horny cell.\textsuperscript{30} Thus, once the soil is wetted with water, the water soluble components of soil dissolve into it. This water also acts as a hydration agent, which increases stratum corneum’s permeability. Mavon A.\textsuperscript{31} suggested that skin surface lipids, mainly sebum, give the skin surface a hydrophilic character which facilitates diffusion of water soluble components. It is for this reason that soil wetted with water gave better clinical response than when applied as powder itself which was observed in another study conducted by us. (un-published data).

In a series of experiments conducted by several scientists \textsuperscript{32} \textsuperscript{33} to study the pattern of absorption of elements through skin, they have come to a conclusion that concentration of
elements in the bathing solution influences its absorption. Dubarry et al. [34] demonstrated that the maximum penetration of the ions through skin was observed when the solute had low salt rather than high salt, and that the more concentrated the external solution is, the less penetrable through the skin are the ions soluble in it. Significantly less $^{131}$I penetrated the skin from a 30%NaCl solution than from 3% or 1% NaCl solutions. Similar experiments were carried out by Giberton and Cohen. [35] In another study Wahlberg et al. [36], [37] noted that the relative absorption of $^{51}$Cr through the guinea-pig skin increased with increasing chromium concentration to a maximal absorption of 4%, and when the concentration was further increased, the absorption decreased to about 1%. The absolute absorption also increased with increasing chromium concentration, but at a certain concentration, a plateau was obtained. The same finding was confirmed later for $^{89}$SrCl$_2$ and for other various elements of the Dead-Sea. [37], [38] These studies indicate that there exists an optimal concentration of elements in the bathing solutions to allow their maximum penetration through skin. This indication was also observed in our studies and thus we could estimate the optimum concentration of elements in our soils for maximum penetration through human skin. This is explained in detail in the following discussion.

Kerala Brown and Vadodara showed approx. 45% of penetration of calcium into the skin (Table no. 1) while Kerala Black, though very rich in calcium showed only 23% penetration. So was the case with Dwarka which contained more calcium than Kerala Brown but its penetration through skin was less than Kerala Brown. These results were in correlation with findings of researchers mentioned above. Thus, we may infer that the concentration of Calcium between 5-9ug/100mg soil, would show about 45% of penetration through human skin.

Penetration of Magnesium was 10% in Kerala Black, 83% in Kerala Brown, 40% in Dwarka and 40% in Vadodara. The table no.1 shows, Kerala Black contained highest Magnesium content amongst the four but showed least skin penetration. These results were also in co-ordination with findings of Dubarray et al. [32], [33] This indicated that if mud has magnesium in the concentration range of 10-12 ug/100mg soil, 83% of it would penetrate into the skin.

The hypothesis of optimal concentration was also observed in Sodium penetration. Kerala Black (38 ug/100mg soil of Na) showed 92% penetration while Kerala Brown (93 ug/100mg soil Na) showed only 40% penetration through skin. In psoriasis, permeability of cells is increased and extracellular sodium is already high [9] so less penetration of it is desirable for
treating psoriasis. Thus concentration of Sodium as 93±10 or 33±4ug/100mg of soil would be favorable for treating psoriasis which would ensure only about 38-40% penetration.

Kerala Black showed 81% penetration of potassium (3.16ug/100mg soil of K), Kerala Brown, 9.7% (10.5 ug/100mg soil of K), Dwarka, 10.5% (5.7ug/100mg soil K), and Vadodara showed 27.7% (2.16 ug/100mg soil of K) penetration of potassium into the skin. (Table no.1). Here too it was observed that if concentration of potassium was in the range of 3-4 ug/100mg in soil then we would have about 81% penetration of it in skin. But since potassium levels are already high in psoriatic skin, soil having concentration of it greater than10ug/100mg of soil, would be preferred because this would lead to approx. only 10% penetration of potassium. Clinical examinations showed that psoriasis patients treated with Kerala Brown showed much better recovery.

There was substantial amount of penetration of phosphorus into the skin from all the four muds. Kerala Black (0.26 ug/100mg soil of P) showed 84% penetration , Kerala Brown (0.163 ug/100mg soil of P) showed 73% penetration, Dwarka (0.332ug/100mg soil of P) showed 45% ,while Vadodara (0.501ug/100mg soil of P) showed only 24% of penetration. Phosphorus is found to be excess in psoriatic skin and is responsible for itching which is experienced by the patients, hence mud which has low penetration of phosphorus would be desirable to treat this disease as seen theoretically. Clinical observations showed that patients who applied Kerala Black complained about itching (which may be attributed due to pH and P penetration (84%)), but those who applied Vadodara and Dwarka did not mention about itching due to mud. Patients who applied Kerala Brown mentioned about mild itching sometimes but overall recovery was seen better with Kerala Brown application.

This study was conducted on healthy, freshly excised abdominal human skin. In psoriasis, skin permeability is increased \cite{39,40,41} and so the penetrability of these elements (Ca, Mg, Na, K, P) may increase in psoriatic patients. This may alter the hypothesized optimum concentration of elements required in soil for therapeutic penetration.1

Our aim of study was to use physicochemical properties of soil to predict its efficacy against psoriasis. Thus, the results of penetration of elements indicated that penetration of Ca and Mg was highest in Kerala Brown. As these are two important elements involved in cell regulation in any inflammatory disease, it implied that this mud would exhibit good clinical efficacy against psoriasis which was confirmed by the results of preliminary clinical studies.
Moreover Flusser D \cite{42} has demonstrated that minerals depleted mud does not give good pharmacological actions against osteoarthritis, indicating that certain concentration of minerals (elements) is necessary for its action. Our study has also demonstrated that optimum concentration of certain elements in mud is necessary for its good therapeutic penetration into skin to exhibit clinical efficacy.

**Humic acid**

It was observed that approx.1.9\% of humic acid of Kerala Black and approx. 1.6\% of humic acid of Kerala Brown soil was retained in 1sq.cm area of human skin. Such low penetration of humic acid may be due to its high molecular weight. Pharmacological action of such low concentration of humic acid (1.6\% / 1sq.cm skin = 34.57ug/1x10^5 cells = ~70ug/2x10^5 cells) cannot be denied because it has been reported that humic acid in concentration range of 50-400ug/ml induces apoptosis in human premyelocytic leukemia (HL-60, 2x10^5cells/ml).\cite{43} Concentration of Humic acid in Dwarka and Vadodara soil was very less (0.086 and 0.076 \% w/w of soil), its penetration into skin was beyond detection limits of our analytical technique.

**Preliminary clinical studies**

Preliminary clinical studies’ results were analyzed by statistical test (ANOVA p<0.05) and it signified that all the four muds were effective in treating psoriasis. Photographs of diseased lesions of patients were taken before starting the treatment and also after 15 days. (Fig.no. 1, 2, 3, 4) Improvement in the symptoms was clearly noticed.

It is to be noted that the patients allotted to us, by the dermatologists of SSG and Ayurvedic hospital, for treatment, were “failure cases” according to them, with long term repeated history of the disease. Nevertheless, we could observe improved clinical symptoms in such patients.1

At the end of 15 days, there was at least 50\% reduction in PASI score which is a clinically significant end point in the assessment of psoriasis.\cite{44} It was observed during the treatment that itching was the first symptom which was improved within 2 days of application. Then gradually, desquamation occurred and then the plaque thickness decreased. This phase of reduction of plaque thickness was a long process (extended beyond 15 days) but during first fifteen days, there was no eruption of any new patch and there was complete eradication of itching. Depending upon the intensity of the disease, it took around 2 to3 months for the skin to gain its normal integrity.
All the patients admitted that there was feeling of coolness on application of mud and this pleasant feeling helped reduce mental tension. Mental stress is one of the causes of psoriasis.\cite{45}, \cite{46}, \cite{47} There was at least 50% reduction in score at the end of 15 days, (initial recovery rate was faster) but it was observed that when the treatment was continued further, there was gradual improvement in the symptoms. The psoriatic lesion was healing from the periphery towards the central portion and the central part (thick plaque) took very long time to heal, depending on the history of the disease.

When another statistical paired t test (for ranking) was applied to clinical data of each mud, for ‘t’ critical value of 1.72, the ‘t’ statistical value was 1.95 for Black, 2.99 for Brown, 2.35 for Dwarka, and 2.86 for Vadodara. Thus, statistical analysis and clinical observations demonstrated Kerala Brown as best mud amongst the four for therapeutic use and Vadodara ranked second.

![Fig. no.1 Psoriasis before treatment. Vadodara mud](image1)

![Fig. no. 2 Psoriasis after treatment (reduction in plaque thickness) (Vadodara mud)](image2)
CONCLUSIONS

The aim of the present investigation was to correlate the physicochemical properties of four different types of muds with their efficacy to treat psoriasis. During our study, it was observed that Kerala Brown mud was most significantly effective in healing clinical symptoms of psoriasis amongst the four muds. Kerala Black mud was also effective, but because of its slightly acidic pH, patients complained about irritation at the first instance of application. Clinical results as shown by the muds could be assigned in the order Kerala Brown > Vadodara > Dwarka > Kerala Black which coincided with statistical analysis and with the degree of penetration of calcium and magnesium into the skin. The possible reasons behind the observed results may be attributed to the difference in their physicochemical parameters. Neutral pH of Kerala Brown played significant role in relieving itching, the first
sign of improvement as compared to Kerala Black. Electrical conductivity data showed that Kerala Black had highest EC amongst the four muds. EC is correlated with soluble salt concentrations of Na, K, Cl, NO₃, SO₄, & NH₄ ions which do not play significant role in healing psoriasis. We could not establish any correlation between electrical conductivity data of mud and skin penetration of elements (Na & K) but in our other study whose data is not published here, electrical conductivity of mud could be correlated to its antimicrobial activity, which in turn could have been one of the factors responsible for healing psoriasis. We could hypothesize optimum concentrations of elements like Ca, Mg, Na, K, P in mud for therapeutic penetration into skin, from our study. The results showed that Kerala Brown had maximum penetration of two important elements namely calcium and magnesium related with psoriasis pathology and probably it may be due to this that Kerala Brown exhibited best clinical efficacy amongst the four muds. If we extend our findings to correlate with colour of soil then we may predict that soil which is blackish brown or brownish red in colour, having approximately neutral pH and EC in the range of 1-5mS, and having calcium and magnesium concentration in the range to ensure maximum therapeutic penetration through skin, may elicit positive pharmacological effects on psoriasis.

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