FTIR AND CARBON ANALYSIS AS TOOLS FOR MONITORING CLINICAL RESPONSE TO MUD THERAPY IN PATIENTS SUFFERING FROM SKIN DISORDERS: CORRELATION OF CHEMICAL AND CLINICAL DATA

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ABSTRACT

Many studies have been conducted worldwide on muds for their dermatological potential to treat psoriasis, eczema, and acne in humans. As no such studies were reported for Indian muds, we chose four topographically different Indian muds, applied them on patients suffering from psoriasis, eczema and acne and tried to correlate the chemical data with the clinical findings. Four different samples of muds were first scanned for FTIR spectra and analyzed for its carbon content by different methods. They were then applied on affected part of skin in patients with psoriasis, eczema and acne. The applied mud was then removed and once again analyzed by FTIR and carbon content by same methods and the disease symptoms were evaluated clinically. Mud is a very good adsorbent and it is reported that the above mentioned skin lesions show over expressions of some of the trigger compounds containing carbon, like free arachidonic acid, phosphoesters, sebum etc. It was observed that there was statistically significant increase (p<0.05) in concentration of carbon content in mud after application and there were changes in FTIR spectra and their intensities, related to the chemical structures present in free compounds like arachidonic acid etc., along with improvement in disease symptoms, possibly indicating adsorption of some skin compounds by mud which helps in healing the diseases. Hence we hypothesized that mud therapy could be effective owing to the removal of such compounds from skin due to adsorption by mud.
KEYWORDS: FTIR, carbon content, muds, skin diseases, adsorption.

(1) INTRODUCTION
Mud (wet soil) has been associated with human beings right from the origin of life. Presence of mud goes synonymously with the presence of life. Scientists have been painstakingly analyzing soil samples from the Moon and Mars, searching for traces of origin of life. Indian Vedic literature has professed that man is born from mud and disintegrates into mud after death (Panchmahabhut). In many cultures like Christian and Islamic cultures, dead bodies are buried in mud and if the grave is dug after a year then, only remnants of skeleton is obtained from it, meaning to say that flesh, skin, organs all disintegrate and merge into mud. Thus it implies that our body is made up of constituents of mud which on decaying become a part of it.

Mud is found to contain minerals like montmorillonite, kaolin, calcite and many groups of organic compounds of nonspecific nature like pentose, cellulose, oxalic acid, succinic acid, lignoceric acid, mannitol, salicylaldehyde resins, trimethylamine, choline, arginine, humic and fulvic acids. However there is inherent difficulty in studying organic compounds of an individual nature because they only occur in small amounts in the soil. But it cannot be assumed that, because these compounds occur in small amounts in the soil, they are of lesser interest, as many of their functions as antibiotics and vitamins are manifested only when they are applied in small amounts.

Psoriasis is a chronic disease with multiple genetic and environmental factors involved in it. There are many exogenous and endogenous factors which may precipitate or aggravate psoriasis. Amongst the endogenous factors, one pathway points towards the disruption in the normal cutaneous biochemistry, and another towards immunomodulatory changes. The resultant pathological condition reveals unregulated proliferation and differentiation of epidermal cells which results in overexpression of many biological components like arachidonic acid, polyamines, eicosanoids, Interleukin etc.

Eczema is an inflammatory skin reaction characterized histologically by spongiosis with varying degrees of acanthosis, and a superficial perivascular lymphohistocytic infiltrate. Factors responsible for this type of skin manifestation include genetic, skin barrier dysfunction, immunological, infection and environmental. As a result there are changes in
various pathological and biochemical processes in skin like overexpression of Interleukins, Prostaglandin, up regulation of stratum corneum chymotryptic enzyme etc.

Acne vulgaris is characterized by noninflammatory, open or closed comedones and by inflammatory papules, pustules, and nodules. The key factor of pathogenesis is genetics. Other factors being hormones, Propionibacterium acnes infection, immunological, and over functioning of sebaceous glands. As a result there is overexpression of certain molecules like Interleukin, matrix metalloproteinase in skin cells.

Therapeutic use of mud has been a tradition not only in India but in Germany, Italy, Israel, Russia etc. Dead Sea mud is reported to be used as a therapy for psoriasis in Israel. Comacchi C reported that a single mud application in patients suffering from seborrhoeic dermatitis improves the values of stratum corneum hydration, TEWL (Total epidermal water loss), skin surface pH and sebum content which are important parameters for integrity of the skin. Single mud pack application has shown to increase skin microcirculation markedly. Russians have also explored therapeutic efficacy of their mud on psoriasis. Mud has been found to modulate production of interleukin-1, decrease TNF-α and increase insulin growth factor. Starichkov AA has observed that mud eliminates intracellular products of inflammation. Korean sea mud has been found to inhibit PGE2 production.

Mud is used as a therapeutic agent for skin diseases in Nature Cure Centers in India as evidence based therapy. Naturopathy believes that mud extracts out the toxins from human body thus helping it to heal. So it was our humble effort to investigate on such toxic compounds by applying preanalysed mud on diseased skin patches and then reanalyzing it to find any change in its constituents. As all the three diseases showed overexpressions of trigger molecules which were bio- organic compounds we chose to monitor the changes in carbon content of muds by methods like, FTIR( Fourier Transform Infrared Spectroscopy), SEM-EDS (Scanning Electron Microscopy with Energy Dispersive Spectroscopy) studies, CHNS(Carbon Hydrogen Nitrogen Sulphur) analysis, and organic carbon content by chemical digestion method. As no previous studies have been undertaken to correlate the chemical composition of the mud and its clinical efficacy, we aimed at using changes in FTIR and carbon content in mud, before and after treatment in patients suffering from skin disorders as markers for predicting the therapeutic response of mud therapy.
(2) MATERIAL AND METHODS

2.1 Collection of mud
Four samples of mud traditionally used for therapeutic and cosmetic purposes were collected from 3 different geographical regions of India. 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, grounded, 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. Soil was wet with water to make paste like consistency, sun dried and dried soil was then collected in a autoseal plastic bag for further investigations.

2.2 FTIR spectroscopy
Fourier Transformed Infrared spectroscopy (FTIR) of the mud was performed on FTIR 8400 S (Shimadzu) at Choksi Laboratories (Vadodara, India) using KBr disc and their spectra were interpreted for characteristic peaks.

2.3 SEM-EDS
Determination of carbon content of mud was done by Scanning Electron Microscope (SEM) attached with LEO 435 VP SEM with Oxford ISIS 300-EDS (Energy Dispersive System) equipment at Metallurgical Dept. M.S.University of Baroda (Vadodara, India). Soil samples were placed on carbon studs and scanned for its carbon content.

2.4 CHNS analysis
CHNS analysis was conducted on CHNS analyzer (Thermo Finnigan Model EA 1112) at IIT Bombay (Mumbai, India). Weighed sample was placed on a disc and combusted. The combustion products were separated by chromatographic column and detected by the thermal conductivity detector.

2.5 Chemical digestion method
Organic carbon content was determined by oxidation of mud’s organic matter by dichromate-sulphuric acid mixture and the intensity of the green colour of the chromium sulphate formed, was measured as amount of carbon oxidized. 200 mg of mud (passed through sieve size 44#) was accurately weighed and transferred to a 250 ml clean and dried volumetric flask. 10 ml of 1N K₂Cr₂O₇ (freshly prepared) was added and shaken a little followed by addition of 20
ml. of concentrated sulphuric acid, shaken again and kept for 30 minutes. Then the contents were centrifuged at 2000rpm for 5 min. and filtered. The green chromium sulphate colour of the supernatant layer was read in the UV-1601 spectrophotometer (Shimadzu) at 660nm adjusting against the blank solution prepared in the same manner without mud.[20]

2.6 Preliminary Clinical Studies

These studies were conducted at the 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, in eczema, EASI (Eczema Area Severity Index) and in acne IGA (Investigator’s Global Assessment) was noted. Patients’ feelings of well-being were also noted down.

A protocol for the study was prepared and Informed Consent Form was filled up by the volunteers. A Case Report was also prepared wherein the weekly observations and therapeutic response of patients were recorded. Photographs of the affected part were also taken during periodical examination.

In all 60 patients enrolled for the study either suffered from psoriasis, eczema or acne and were of the age group between 18 to 60 years. Five subjects were used in each disease group (psoriasis, eczema, acne) for each mud sample. The study was continued for 3 months during which samples were collected every 15 days and analyzed for its carbon compounds. The patients were asked to wet the soil and apply on the affected part and leave it on the skin for 30 minutes once daily. After 30 min., mud was gently scrapped off the application site and collected in a clean dry plastic bag. Samples of mud collected from normal healthy persons who also applied mud on the ventral part of their forearms were used as controls. FTIR spectroscopy was performed on the collected samples and analyzed with respect to their carbon content by SEM-EDS, CHNS analyzer and chemical digestion method as described previously.

RESULTS AND DISCUSSIONS

3.1 FTIR study

Fourier Transform Infrared (FTIR) spectroscopy is a commonly used technique capable of distinguishing the principal chemical classes in soil organic matter, such as carbohydrates,
lignins, cellulose, fats and/or lipids, waxes, carboxylic acids and proteinaceous compounds, through the vibrational characteristics of their structural chemical bonds.

On comparing the infrared absorption data of all four muds applied on diseased part of patients with that of mud applied on healthy persons and with original mud, as shown in figures no.1, 2, 3, and 4, we observed several new peaks in many band regions and also changes in intensities of their existing peaks. Moreover several peaks which existed in original mud were lost. This implied that both adsorption of skin contents by mud and absorption of mud constituents by diseased skin were occurring.

Mud is a strong adsorbent. The clay part of mud is reported to adsorb large number of compounds by various adsorption mechanisms like hydrogen bonding, Ion exchange, physical adsorption (Vander Waals forces), coordination (non-ionic adsorption) and chemisorption.[21] Therefore, we tried to correlate the changes occurring in the spectra of MAP (Mud applied on patients), normal persons and original mud with the reported pathology of diseases.

The following sections detail the various processes and reasons for their interpretations. Kindly refer all the four figures for explanations.

Mortland MM[22] observed that water molecules in the primary hydration shell of cations adsorbed on clay can be displaced by alcohols. This exchange was observed by increased intensity in absorption bands in 3670-3580 cm\(^{-1}\) range, the range of OH stretching vibrations of alcohols, and new peaks in [fig.1] Kerala Brown, psoriasis: 3601, 3622 eczema: 3622, acne: 3601,3622 cm\(^{-1}\). In [fig.2] Kerala Black, psoriasis: 3620 eczema: 3620 acne: 3620 cm\(^{-1}\). In [fig.3] Dwarka, psoriasis: 3587, 3620 eczema: 3618, 3628 acne: 3628 cm\(^{-1}\). In [fig.4] Vadodara, psoriasis: 3618 eczema: 3626 acne: 3599, 3618, 3620, 3626 cm\(^{-1}\). Excessive activity of sebaceous glands is considered as one of the causes of acne and sebum is composed of squalene, wax esters, sterol esters, free fatty acids, sterols, glycerides and saturated hydrocarbons.[23] Appearance of peaks representing functional groups of these constituents of sebum indicates the possibility that mud may have adsorbed sebum which would have contributed towards improvement in acne symptoms as evidenced by the clinical findings discussed later.
Excess phosphorus is a causative factor for itching (acting through Vit.D channel).\textsuperscript{[24, 25]} Decrease in itching was the first improvement symptom observed in all the three diseases especially in eczema and psoriasis. 2300-2600 cm\textsuperscript{-1} is also the region of P-OH and P-H stretching vibrations and there are several new peaks in this region in MAP of all the three diseases like In [fig.1] Kerala Brown, eczema: 2322, 2363, 2415, 2434, 2452, 2469, 2486, 2506, 2523, 2542, 2562, 2581, 2598; acne: 2324 cm\textsuperscript{-1}. In [fig.2] Kerala Black, Psoriasis: 2349, 2393, 2409, 2430, 2447, 2482, 2497, 2515, 2536, 2553, 2575 eczema: 2347, 2384, 2399, 2418, 2436, 2470, 2490, 2507, 2526, 2544, 2582 acne: 2399, 2451, 2470, 2505, 2578, 2617 cm\textsuperscript{-1}. In [fig.3] Dwarka psoriasis: 2330, 2360, 2522 eczema: 2312, 2361, 2523 acne: 2312, 2330, 2359, 2522 cm\textsuperscript{-1}. In [fig.4] Vadodara psoriasis: 2310, 2328, 2343, 2359, 2467, 2538 eczema: 2359 acne: 2312, 2328, 2359, 2533 cm\textsuperscript{-1}. 770-790 cm\textsuperscript{-1} corresponds to P-CH\textsubscript{3} stretching vibrations and there was change in intensity at 779 cm\textsuperscript{-1} in MAP of all the four muds applied on all 3 diseases. Thus appearance of new peaks and change in absorption intensities in P-OH, P-H, P-CH\textsubscript{3} stretching vibration regions indicate adsorption and thus removal of phosphorus compounds by mud and this may probably explain the improvement in itching symptoms as evidenced during clinical studies.

Psoriasis is a disease of irregular proliferation of epidermis \textsuperscript{[26]}. Humic acid exhibits an antiproliferative effect by inducing apoptosis that is associated with cytochrome C translocation, caspase 3 activation, degradation of PARP (poly ADP-ribose polymerase) and dysregulation of Bcl-2 and Bax protein in HL-60 cells \textsuperscript{[27]}. Humic acid mainly contains ketone and aldehyde groups. Initially, adsorption sites in a soil system can be considered to be occupied by water, and adsorption of an organic molecule usually involves desorption of Type II (loosely bound) water from the colloid surface \textsuperscript{[28]}. The decrease in the intensity of absorption peak (2312 cm\textsuperscript{-1}), of humic acid (2310-2350 cm\textsuperscript{-1}) in psoriasis and loss of peak in eczema in MAP, indicates a possibility that humic acid may have desorbed from the soil and diffused into the skin. Report of humic acid’s role in apoptosis of HL-60 cells led us to conclude that reduction in the plaque thickness observed clinically in psoriatic and eczematous patients may be attributed to the penetration of humic acid into the skin. (Presence of humic acid in skin during ex-vivo diffusion study confirmed this phenomenon).

Free Arachidonic acid cascade is one of the major cause of triggering fast cell proliferation and inflammation which occurs in psoriasis \textsuperscript{[29]}. Eicosanoids play an important role in skin inflammatory diseases like psoriasis, eczema and acne also \textsuperscript{[29]}. The carboxyl group of the organic acid interacts either directly with the interlayer cation or by forming a hydrogen bond.
with the water molecules coordinated to the exchangeable cation on the clay complex. In addition to coordination and hydrogen bonding, organic acids can be adsorbed by forming salts with the exchangeable cations \[^{30}\]. The absorbance intensities of band 912.36 cm\(^{-1}\) which is in the range of 800-920 cm\(^{-1}\) (C-O stretching vibration and O-H vibration of carboxylic acid,) as well as absorbance at 694.4 cm\(^{-1}\) (attributed to Mg/Al and R\(_1\)CH=CHR\(_2\)) increased in all the four muds applied in all 3 diseases, which indicated that adsorption of free acid present in diseased skin \[^{4,29}\] might be taking place by OH bonding with exchangeable cations like Mg\(^{++}\), Al \(^{+++}\) (Mineralogical analysis). 1630-1650 cm\(^{-1}\) range is due to C=O stretching vibrations of carboxylic acid and absorbance intensities of all four MAP increased in this range, which prompted us to conclude that free arachidonic acid or other free eicosanoids present in diseased skin may be adsorbed by the soil, (thus removed from diseased lesion), as a result of which thinning of plaques may have occurred.

Amines can protonate in soil and can replace inorganic cations from the clay complex by ion exchange. Uncharged amino acids and peptides can be adsorbed physically \[^{31}\]. When amines form double-layer complexes, there is a weak hydrogen bonding between the NH\(_2\) group of the amine and oxygen on the silicate surface \[^{32}\]. Absorbance at 1031.95 cm\(^{-1}\) (silicate bonding and N bonding) in all four muds applied on all 3 diseased skins was greater than that in mud applied to control’s skin. Hence we could draw the conclusion that amines or compounds containing nitrogen (eicosanoids, arachidonic acid) must have got adsorbed on the soil from diseased skin.

Total carbon is associated with the main polysaccharide envelopes at 1030 and 3300 cm\(^{-1}\), lignin like compounds (1513, 1450, 1371, 1265, and 835 cm\(^{-1}\)) and aliphatic structures at 2920 and 2850 cm\(^{-1}\) (fats, waxes and lipids). Spectra of MAP of all the three diseases, exhibited increase in absorption at these frequencies indicating increase in total carbon content. (This was also confirmed by increase in carbon content in MAP when analyzed by CHNS, SEM-EDS and chemical digestion method).

Hence we could deduce that emergence of new peaks and increase in intensity of IR absorption of functional groups of the carbon containing compounds like sebum (carboxylic acids, alcohols, esters, waxes), phosphocarbons (P-CH\(_3\), P-OH, P-H), amines, arachidonic acid, eicosanoids (unsaturated fatty acids) which play role in pathogenesis of these diseases (psoriasis, eczema and acne), get adsorbed on the MAP and get removed from the diseased skin patch. Moreover, simultaneous improvement in the disease symptoms like reduction in
plaque thickness, relief in itching, reduction in redness etc. in all the three diseases, was observed clinically. This led us to conclude that removal of these carbon containing pathogens from diseased skin was directly correlated with its recovery. It is reported that when free arachidonic acid is released in the epidermis and elsewhere (i.e. in blood vessels, platelets, leukocytes and dermis), a specific array of biologically potent lipids and mediators of inflammation like LTB4 (leukotriene B4) and 12HETE (12 hydroxyeicosatetraenoic acid) in case of psoriasis can be identified [33]. Hence presumably removal of free arachidonic acid from the skin which is produced as a disease process, limits its further transformation to LTB4 and 12 HETE which are hypothesized to play significant role in producing psoriatic lesions, causes improvement in the disease. Improvement in psoriasis by Benoxaprofen by inhibiting LT formation and by adrenal steroids by inhibiting the release of arachidonic acid is well known [34]. Thus one of the mechanisms of action of mud in healing these proliferative inflammatory skin diseases may be by removal of these toxins from the skin surface through adsorption by the applied mud.

Itching was a common symptom of psoriasis, eczema and acne. It is established that Vit.D and its analogs controls calcium and phosphorus metabolism thus treating itch, rash and angioedema. [35]. In another study, a 31P magnetic resonance spectra of psoriatic skin showed that there was elevations in phosphomonoester concentrations and phosphomonoester/phosphodiester ratio and in conjunction with chromatographic analysis, it showed a defect in phosphometabolism [24]. Phosphorylase kinase activity was also elevated in psoriatic epidermis [25]. We have explained earlier about emergence of many new peaks in P-OH, P-H, P-CH₃ vibration band regions. This indicates that removal of excess of phosphorus from diseased skin surface by adsorption by mud may have played a role in reducing itching and rashes experienced by the patients. This was confirmed when the patients were observed clinically.

To support the above data of adsorption of carbon containing compounds, other quantitative tests were also performed.

3.2 SEM-EDS Study

In this study carbon content was measured by energy dispersive techniques. This technique separates and detects X-rays of specific energy emitted by the atom when bombarded by electron beam and displays them as histograms. The data in table no. 1 indicated that the percentage of total (organic and inorganic) carbon content detected in mud applied on
psoriatic, eczematous and acne lesion was significantly higher (p<0.05 ANOVA) than that of control group. This confirmed that mud had adsorbed carbon compounds from the surface of the diseased skin.

Table no 1: % carbon content of mud applied on diseased skin by SEM-EDS method

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Mud</th>
<th>Original mud</th>
<th>Control group</th>
<th>Post applied</th>
<th>Psoriasis</th>
<th>Eczema</th>
<th>Acne</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kerala Black</td>
<td>3.78±0.01</td>
<td>3.97±0.09</td>
<td>11.61±0.40</td>
<td>8.96±0.90</td>
<td>5.62±0.70</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Kerala Brown</td>
<td>4.89±0.03</td>
<td>7.08±0.08</td>
<td>18.66±0.30</td>
<td>16.58±2.0</td>
<td>10.22±0.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Dwarka</td>
<td>5.66±0.02</td>
<td>7.41±0.10</td>
<td>20.73±0.90</td>
<td>18.41±1.0</td>
<td>13.60±1.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Vadodara</td>
<td>2.17±0.01</td>
<td>2.3±0.08</td>
<td>10.96±0.8</td>
<td>8.97±0.9</td>
<td>5.98±0.8</td>
<td></td>
</tr>
</tbody>
</table>

3.3 CHNS analysis

Another method, CHNS analysis which measures total dry combustible carbon in the form of CO\(_2\) was also adopted to determine adsorption of carbon compounds from diseased skin. Organic compounds are generally combustible at 1800°C and so this technique measures organic carbon content quite precisely. The figures in table no. 2 indicated that there was significant (p<0.05 ANOVA) rise in carbon content in muds applied on psoriatic, eczematous and acne lesions.

Table no.2: Concentration of Carbon in percentage as measured by CHNS analyzer

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Mud</th>
<th>Original Mud</th>
<th>Control group</th>
<th>Post applied</th>
<th>Psoriasis</th>
<th>Eczema</th>
<th>Acne</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kerala Black</td>
<td>2.761±0.05</td>
<td>2.760±0.02</td>
<td>3.230±0.06</td>
<td>2.940±0.10</td>
<td>3.110±0.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Kerala Brown</td>
<td>0.717±0.04</td>
<td>0.930±0.01</td>
<td>1.290±0.06</td>
<td>0.955±0.08</td>
<td>1.400±0.04</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Dwarka</td>
<td>8.898±0.06</td>
<td>8.379±0.60</td>
<td>9.730±0.3</td>
<td>8.966±0.40</td>
<td>9.520±0.08</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Vadodara</td>
<td>0.289±0.02</td>
<td>0.398±0.05</td>
<td>0.560±0.05</td>
<td>0.497±0.03</td>
<td>1.060±0.02</td>
<td></td>
</tr>
</tbody>
</table>

3.4 Chemical digestion method

In this method oxidation of mud’s organic matter is carried out by dichromate-sulphuric acid mixture and the intensity of the green colour of the chromium sulphate formed is measured to give directly the amount of carbon oxidized. Thus this method measures only oxidisable organic carbon and so the percentage content of carbon is far less than measured by the previous two methods. Nevertheless, the figures in table no.3 indicated that there was significant increase (p<0.05 ANOVA) in the oxidisable organic carbon content of mud applied on diseased patches of skin.
Thus, inspite of variation in the principles of measuring carbon content by different methods and equipments results obtained by all the 3 methods indicated that mud applied on diseased skin had more carbon content than that of mud applied on control group and that in original mud. These results further strengthened our hypothesis that muds helped in the treatment of skin disorders by adsorbing carbon compounds.

### 3.5 Clinical studies

Initial and final score of PASI, EASI and IGA evaluation methods for psoriasis, eczema and acne respectively were calculated for the first 15 days of treatment and were correlated with results of carbon content determination. Applying ANOVA to this data, it was observed that there was significant (p<0.05) difference in the initial and final scores, signifying that application of mud on skin lesions of psoriasis, eczema and acne was clinically beneficial.

Almost all patients experienced reduction in itching as the first effect on applying mud. Moreover, since mud was wet, it gave cooling effect which gave pleasant feeling to the patients. The subsequent effect on the skin was thinning of the plaque in case of psoriasis, stoppage of oozing of fluid from eczematous lesions and starting of suppression of comedones, and decrease in the oiliness of skin in acne.
Fig No: 01 Infra-red spectroscopy of Kerala Black Mud applied on diseased skin.

Fig. No. 02 Infra-red spectroscopy of Kerala Brown Mud applied on diseased skin.
CONCLUSIONS

FTIR data shows the emergence of new peaks in the region of functional groups of trigger compounds like arachidonic acid, sebum, phosphocompounds etc. reported to be present free...
on the skin lesions of psoriasis, eczema and acne patients. Out of these, role of free fatty acids like arachidonic acid in causing proliferative inflammatory diseases like psoriasis, is well established [29]. Excess of sebum is also one of the causes of acne [11]. Disrupted phosphorus metabolism in these diseased cells is also reported [24]. Loss of humic acid peak in eczema and decrease in its (peak) intensity in MAP in psoriasis also indicates penetration of humic acid into the diseased skin. New peaks, loss of peak, and increase and decrease in intensities of some existing peaks observed in FTIR spectra related to these types of trigger compounds has been elaborately discussed. Moreover FTIR data is also supported quantitatively by SEM-EDS, CHNS and chemical digestion studies. Thus it is established that carbon content of mud applied on diseased skin is significantly increased compared to that of mud applied on control group’s skin. The increase of carbon content, along with improvement in disease symptoms like reduction in itching, and plaque thickness, improvement of skin integrity in eczema, suppression of pimples in acne etc., as noticed clinically, directly indicated that removal of carbon containing substances from diseased skin may play a role in its recovery. Hence FTIR and carbon content analysis of mud applied on skin lesions of psoriasis, eczema and acne may be utilized as a tool to monitor the clinical response of the disease and a day may not be far when a marker compound will be identified to monitor the prognosis of these proliferative inflammatory diseases.

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