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Extraction of Clay from Red Soil and Evaluation of Its UV Protection Properties

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Abstract: ***Clay has been used for centuries to beautify the skin. Clays are used as a face mask, bleaches, scrubs and also incorporated in natural soaps. It has special use in herbal spas and used as a curative medicine in Ayurveda. Apart from this, Minerals present in clays are administered orally to the patient which acts as mineral supplements, antacids, gastrointestinal protectors, antidiarrhoeaics, oral laxatives, direct emetics, antianemics or homeostatics. Clay has many properties of medicinal and cosmeceutical values. Indian soil and its clay also has so many beneficial capabilities. The Himba community of Namibia applies Red clay from head to toe to protect their skin from harmful UV radiations. But the proper evaluation of Indian red clay for its UV protection property was not yet done. For this purpose, Indian clay from Red soil was evaluated for its UV protection properties. Soil was collected and clay was separated from the soil. It was evaluated to determine its suitability as cosmetic ingredient for UV protection capability. In the present study, clay was extracted from red soil and evaluated for its Sunscreening activity.***

Keywords: Ultraviolet radiation, Sun protection Factor, clay, soil, microbial evaluation

1. Introduction

India is an agricultural country. Soil is an important natural resource for India and they form almost 45.6% agricultural area. Soil is very important to human and all living creatures which are dependent on soil for food, shelter, cloth and medicine which directly or indirectly coming from soil. Soil is the source of minerals for treatment of various incurable diseases. It can be used as antacids, gastrointestinal protectors, antidiarrhoeaics, oral laxatives, direct emetics, antianemics or homeostatics.

Clays such as fuller's earth, bentonite, etc has been widely used in cosmetics as it detoxifies the skin, absorbs excess oil and refreshes the skin. Various other cosmetic clays have been used for various skin types according to their respective uses. Clays are also utilizing for the purpose of beautification and cleansing. Now a day, clays are incorporated in Shampoos and Soap to absorb excess oil and also to enhance the lathering property of the cosmetics. Face mask, skin bleaches and scrubs are usually formulated. Clays is used since ancient time for maintaining the skin condition and now-a-days it is used in luxury spas.

Clays can have other cosmetic values but proper study was not yet done. From the literature, it was found that the Himba community of Namibia covered their body from toe to head with red clay which protects their body from the harmful UV radiations. Therefore various studies were carried out in Africa to study the effect of this red clay as potential UV protective agent. [1]

Red soil is present in India in abundance which forms almost 10.5% of total land area. Indian red clay can act as a great UV protective agent.

UV Radiation

UV radiation, a type of electromagnetic radiation with wavelengths ranging from 10 to 400 nm, is well known for its harmful acute and chronic effects on the human skin and eye, e.g., sunburn, skin aging, and the extreme case of skin cancer.). On the basis of wavelength, several types of UV radiation are distinguished, with the following being most important: long-wave UV-A (400–320 nm), UV-B (320–280 nm), and short-wave UV-C (280–100 nm) [2]. UV-A is thought to cause skin aging and erythema or sunburn, whereas UV-B may cause DNA damage and skin cancer. UV-C is the highest-energy and most dangerous type of UV radiation, but it is generally absorbed by the ozone layer in the atmosphere (WHO, 2002). [3]

Due to these health effects on the skin many types of sunscreen creams have been formulated and available in market containing UV-protection compounds, including organic and inorganic materials. However, the non-natural substances present in these creams as main UV-protection agents, e.g. micronized TiO2, are known to cause an unexpected photo-catalytic effect, which may be a serious problem on the skin.[4] Therefore, natural materials are being sought as replacements for the synthetic UV protection agents. Potential compounds as natural UV-protection agents in sunscreens include clays and clay minerals, which due to their many benefits for human health are already utilized in various types of pharmaceutical and cosmetic products. [1]

The present study examines the physical, microbial and chemical properties of Indian red clay and provides systematic data for the efficiency of the UV protection capabilities in the UV-A and UB-B spectral ranges.

2. Material and Methods

2.1 Collection of Soil

Red soil was collected from Bhandara, Maharashtra, India. It was collected from the digging area from the 10 inches below the surface of ground. Soil is brick red in colour and has characteristic earthy odour. It is easily available in

Bhandara district because large part of red soil distribution was found in the district.

2.2 Determination of content of clay

- **Requirements**: beaker, graduated measuring cylinder, stirrer.

- **Procedure:**

a) Graduated cylinder was cleaned with water and dried.
b) 100 g of soil was taken in the cylinder and water was poured to make up 100 ml.
c) 5 ml of detergent was added in the soil suspension. Detergent was used to dissolve the soil aggregates and keep the individual soil particles separated. [5]
d) It was vigorously shaken for about 5 minutes until the soil completely dispersed in water.
e) It was allowed to settle untouched for about a week.
f) Readings were recorded with the help of measuring scale.

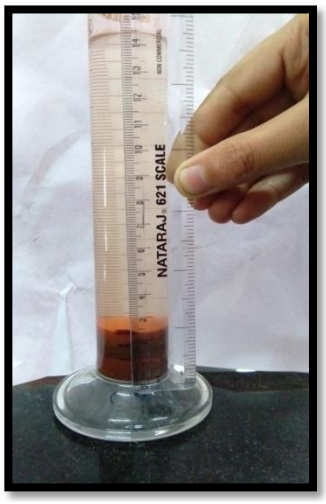


Plate 1: Determination of content of clay

Content of sand =

$$\frac{1.5.\times 100}{2.5}$$

= 60%

Content of slit =

$$\frac{0.7\times 100}{2.5}$$

= 28 %

Content of clay =

$$\frac{0.3\times 100}{2.5}$$

= 12%

2.3 Extraction of clay: The method followed is Water Extraction method.

Requirement: Glass jar, measuring cylinder, cloth, oven, sieve, mortar-pestle

Procedure: Soil was suspended in water for 4 days and upper layer (clay) of the soil suspension was carefully separated from the lower layer (slit and sand). If any amount of clay and slit remained in the clay following procedure was followed:

a) Equal amount of water was taken and 500 gm of soil was placed and was sieved properly from the 63 micron mesh sieve size.
b) Double folded cloth was taken and the complete soil suspension was poured on the cloth.
c) All the water from the clayey soil was removed by hanging it in cloth for at least 1-2 weeks.
d) the clay was dried by keeping it in the oven for 72 hours at temperature of 35-40^{o} C
e) any powder lumps present was crushed and fine powder should be made using mortar and pestle.[6]

Calculations:

Table 1: calculation table for amount of clay

1	Quantity of soil taken	500 g
2	Quantity of water taken	500 ml
3	Amount of clay obtained	85 g
4	Amount of clay obtained after drying	54 g

2.4 Total Microbial count (TMC) of clay sample and Fuller's earth

Due to presence of so many microorganisms in the soil, it is necessary to carry out microbiological evaluation of soil for its intended use in cosmetics as an ingredient. Fuller's earth was taken as standard. Clay and fullers earth was evaluated before Sterilization and after sterilization.

Microbiological assessment was carried out on the basis of the BIS standard. [7]

Materials required: sterile petridish, sterile graduated pipette, sterile volumetric flask, neutralizer (tween 80), inactivating broth, soyabean casein dextrose agar, buffer solution, saboured dextrose agar.

Principle: This method involves the enumeration of colonies on a nonselective agar medium (plate count). The possible inhibition of the microbial growth by the sample shall be neutralized to allow detection of viable micro-organisms.

Procedure: Sterilization of Apparatus: -All the apparatus were cleaned, dried and sterilized. The sterilization was done by wrapping the apparatus in the paper. The autoclaving is done in a hot air oven at 121^{0} C for 1 hour. [7]

Preparation of Media:

1) 65.0 g SDA was dissolved in 1000ml of distilled water. Similarly, 40g of SCDA was dissolved in 1000ml of distilled water
2) It was kept for 20 minutes for proper dissolution of agar by heating on the water bath.

3) The media was transferred into clean conical flask after dissolution with tight fitting cotton plug.
4) It was sterilized in an autoclave at 121°C and lbs. pressure for 20 minutes. [7]

Method

1) 1ml of product and 9ml of peptone water was weighed. It was transferred in sterile test tube.
2) Similarly, 1:100(10^{-2}), 1:1000(10^{-3}) dilutions was prepared from the above samples using same diluents.
3) 1ml of each dilution was aseptically transferred in petridish using sterile pipette for SCDA and SDA media.*
4) The media was quickly poured into the petridishes to avoid solidification of the medium or formation of lumps because of rapid drop in temperature.*
5) The medium was allowed to solidify and petridishes were incubated in an inverted position at 33 ± 2ºC for 48hrs for bacteria and 28 ± 2ºC for 3to 5 days for fungi and yeast. [7] * Procedure modified as required. [7]

2.5 Physical Evaluation

2.5.1 pH

Procedure:

1) pH meter was standardized using a buffer solution of pH 7 and pH 9 before the pH of the sample was determined.
2) The pH was determined for 5% solution of the product.
3) 5 gms of sample was taken in the beaker and was dissolved in 100 ml of distilled water.
4) Sample was mixed properly until properly dispersed.
5) The electrode then dipped into the sample.[8]

2.5.2 Color determination:

Colour determination was done by visually comparing the samples with soil colours in the Munsell Soil Colour Charts to obtain the hue, value, chroma and colour of the samples. [9-12]

2.5.3 Bulk Density:

Laboratory Method: Bulk density can be calculated by using following method:

1. Weighed amount (M) of clay sample was taken in the Measuring cylinder
2. Initial volume was noted (V_1)
3. Cylinder was tapped 50 times using index finger.
4. Final volume after tapping was calculated(V_{50})[13-14]

2.5.4 Texture: Texture of the soil can be studied through Soil Texture Triangle. The percentage of amount of sand, slit and clay was put in the columns of [15] and texture of the soil was determined.

2.5.5 Moisture Content:
Moisture content can be obtained by keeping the weight sample in oven until constant mass. As most of the soil or clay content some amount of water in it, it is necessary that the clay should be free from any water as it will prevent the growth of many types of bacteria. Clay is the rich source of many minerals and therefore the proper removal of moisture is necessary. (16-17)

2.5.6 Particle Size and structure:

Particle size and structure was determined by using **Scanning Electron Microscope** (SEM). [18]. Procedure was carried out in Visvesvaraya National Institute of Technology (VNIT Nagpur).

2.6 Chemical composition of clay:

Main Component of clay can be determined through the technique of X-Ray Diffraction. Powder sample were studied using PANalytical X'pert pro X-ray Diffractometer equipped with a Cu tube (CuKα_{12}radiation) and operated at the current of 40mA and a voltage of 45 kV. [2]

2.7 In vitro efficacy test by UV Measurements:

➢ Reagents and samples

Ethanol (analytical grade).

➢ Apparatus

Shimadzu UV-1800 UV/Visible spectrophotometer, equipped with 1 cm quartz cell, computer and printer.

Methods:

➢ Sample preparation:

1. 1 g of samples was weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol, followed by vigorous mixing for 5 min and then filtered through Whatsmann Filter Paper, rejecting the first 10 ml.
2. A 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol.
3. Then a 5.0 ml aliquot was transferred to a 25 ml volumetric flask and the volume completed with ethanol.
4. The absorption spectra of samples in solution were obtained in the range of 290 to 320 nm using 1 cm quartz cell, and ethanol as a blank.

The absorption data were obtained in the range of 290 to 320, every 5 nm, and 3 determinations were made at each point, followed by the application of Mansur equation. [19]

Formula:

The SPF of natural clay material and control material was calculated by using following formula:

$$SPF = CF \times \Sigma_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where,

CF is the correction factor,
I is the wavelength,
EE is the erythemal effect,
I is the solar intensity spectrum and
Abs is the absorbance of the diluted sample.
Each analysis was performed in triplicate.

The values of EE×I are constants and pre-determined by Sayre et al, And are showed in Table 14

Table 2: Normalized product function used in the calculation of SPF (20)

Wavelength(nm)	EE × I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180
total	1

3. Results

3.1 Microbiological evaluation:

Plate 2: Bacterial count (medium-SCDA) for clay sample after sterilization

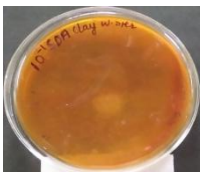


Plate 3: Yeast and mold count (medium-SDA) for clay after sterilization

Calculations: 10^{-1} Dilution**:**

Bacterial count(BC) =1×10^{-1} = 1×10= 10
Yeast and mould count(YMC) = 0×10^{-1}=0

Total microbial count = BC+YMC= 10= 10 cfu/gm
There were no growth observed in the sterilized clay after 10^{-2} dilutions.

Clay before sterilization

10^{-1} Dilution – 10^{-3} dilution: Contamination with large number of bacteria, yeast and mould was observed in the **1:10** dilution. Calculation of TMC was not possible. Overgrowth was observed in further dilutions from 1:100 and 1:1000. Later on, no growth was observed in further dilutions.

Plate 4: Bacterial count (medium-SCDA) for Fuller's earth after sterilization

Plate 5: Yeast and mould count (medium-SDA) for Fuller's earth after sterilization

Calculations: 10^{-1} Dilution:

Bacterial count(BC) =1×10^{-1} = 1×10=10
Yeast and mould count(YMC) =1×10^{-1}=1×10=10
Total microbial count = BC+YMC= 10+10= 20 cfu/g

Note: No Growth was observed in dilution 10^{-2} and 10^{-3}

Plate 6: Bacterial count (medium-SCDA) for Fuller's earth before sterilization

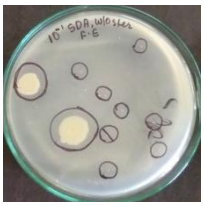


Plate 7: Yeast and mould count (medium-SDA) for Fuller's earth before sterilization

Calculations:10^{-1} Dilution:

Bacterial count(BC) =12×10^{-1} = 12×10= 120
Yeast and mould count(YMC) =12×10^{-1}= 12×10=120
Total microbial count = BC+YMC= 120+120= 240cfu/gm

Note: No growth was observed in the further dilution.

Table 3: Combined results of microbial evaluation

Sr no	TMC, max	For clay	For Fuller's earth
1	After Sterilization	10 cfu/g	20 cfu/g
2	Before Sterilization	Overgrowth	240 cfu/g

3.2 Physical Evaluation

3.2.1 pH:

Table 4: pH of clay and fuller's earth

No	Sample	pH
1	Red clay	6.25
2	Fuller's earth	5.63

3.2.2 Color determination:

Table 5: Colour determination of clay sample used as sunscreen

Hue	5YR
Value	5
Chroma	8

YR=Yellow- Red

3.2.3 Bulk Density: Table 6: Bulk density of clay used as sunscreen

Initial volume of sample	59 ml
Final volume of sample after tapping	47 ml
Weight of sample	51.08 gm

Bulk Density was measured by using following formula:

$$\text{Bulk Density} = \frac{M}{V_1 - V_{50}}$$

Where, V_1 - Initial volume
V_{50} – Final volume after tapping
M – Weight of sample
Bulk density = 51.08/47 = 1.08g/ml

3.2.4 Texture:

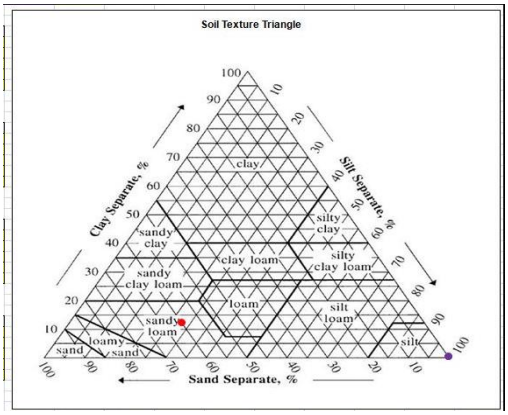


Plate 8: Soil Texture Triangle

Result: Texture obtained is **Sandy Loam** type of soil

3.2.5 Moisture Content:

Observation Table:

Weight of Petri dish: 38.69 g
Temperature of oven: 70^0C

Table 7: Moisture content determination of clay

Time	Weight of sample (before)	Weight of sample (after)
15 min	49.78 g	48.10 g
30 min	48.10 g	47.87 g
45 min	47.87 g	46.56 g
60 min	46.56 g	44.79 g
75 min	44.79 g	42.92 g
90 min	42.92 g	41.97 g
105 min	41.97 g	41.58 g
120 min	41.58 g	41.58 g
135 min	41.58 g	41.58 g

Calculations: % moisture content =weight of sample obtained after removal of moisture/ Weight of the sample taken for the test × 100

$$= \frac{49.78 - 41.58 \times 100}{49.78}$$
$$= 16.47\%$$

Result: % moisture content obtained was 16.47 %

3.2.6 Particle Size and structure: Following results were obtained:

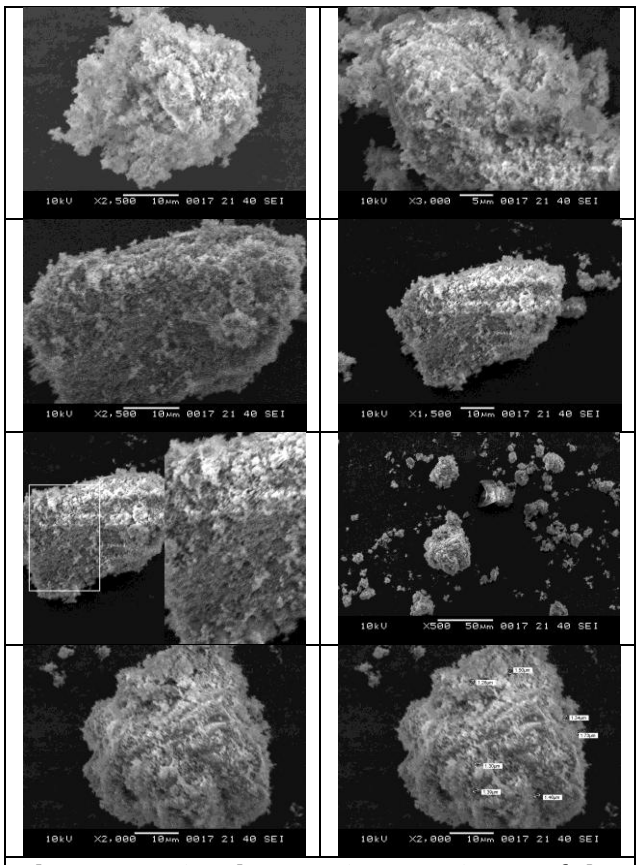
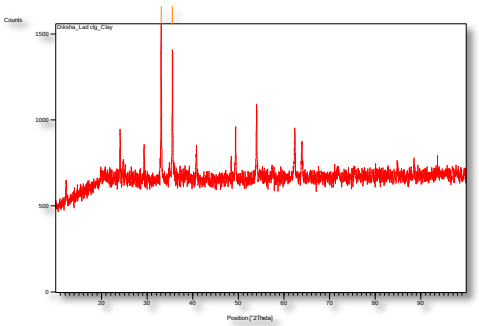


Plate 9: Scanning electron microscopy images of clay sample showing their morphology and particle size

Result: Particle size**:** From the report obtained**,** the particle size was found to be in the range of **1.28µm to 1.73µm.** The average particle size is **1.42µm**

Structure: The structure observed through SEM results is porous with different size ranges.

3.3 Chemical composition of clay: Following results were obtained:



Graph 1: Graph showing XRD peaks

Table 8: peak list of x-ray diffraction

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
33.0644	907.47	0.1511	2.70704	100.00
35.5425	700.97	0.1786	2.52377	77.24

Table 9: pattern list of x-ray diffraction

Visible	Ref. Code	Score	Compound Name	Displacement [°2Th.]	Scale Factor	Chemical Formula
*	01-073-2234	53	Iron Oxide	-0.101	0.986	Fe2 O3

Result: From the report obtained, the major constituent found in the clay sample was Iron Oxide which forms a largest peak on graph. Apart from that some amount of toxic elements such as mercury, lead and cadmium were found during analysis in its compound form.

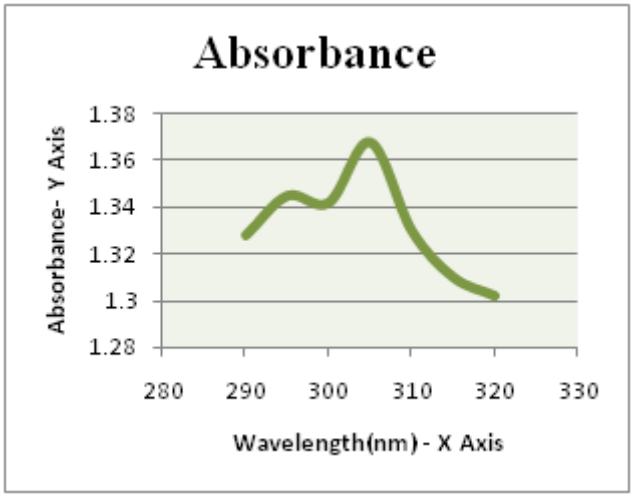
3.4 In vitro efficacy test by UV Measurements:

Analytical instrument conditions to be considered before experimentation.
Instrument: UV- Visible spectrophotometer, UV-1800, Shimadzu
Cuvette: Quartz, 1 cm path length
Wavelength: 290-320 nm
Scan speed: Fast
Slit: 2

Observation and Calculations:

Table 10: SPF calculated for Clay material

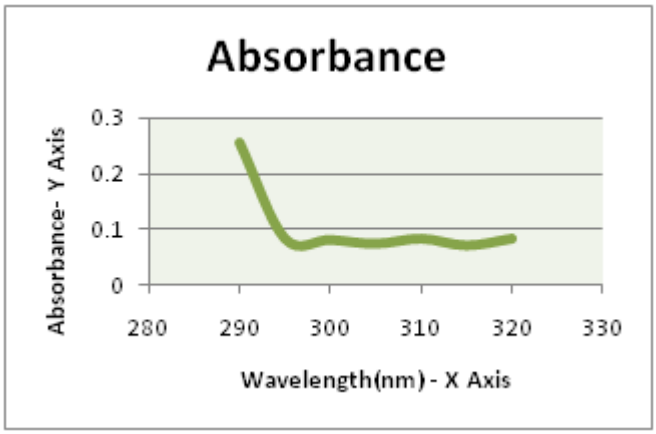
Wavelength(nm)	Absorbance			Mean	EE×I
	I	II	III		
Blank	-	-	-	-	-
290	1.368	1.368	1.368	1.328	0.0150
295	1.345	1.344	1.345	1.345	0.0817
300	1.342	1.342	1.342	1.342	0.2874
305	1.327	1.330	1.328	1.368	0.3278
310	1.330	1.330	1.330	1.330	0.1864
315	1.308	1.310	1.310	1.310	0.0837
320	1.302	1.302	1.302	1.302	0.0180
SPF					**13.451**



Graph 2: Graph showing Absorbance with corresponding wavelength for Clay material

Table 17: SPF calculated for Fuller's earth

Wavelength(nm)	Absorbance			Mean	EE×I
	I	II	III		
Blank	-	-	-	-	-
290	0.256	0.255	0.256	0.256	0.0150
295	0.083	0.083	0.084	0.084	0.0817
300	0.082	0.082	0.082	0.082	0.2874
305	0.075	0.075	0.076	0.075	0.3278
310	0.084	0.084	0.85	0.084	0.1864
315	0.072	0.074	0.072	0.072	0.0837
320	0.084	0.084	0.084	0.084	0.0180
SPF					**0.814**



Graph 3: Graph showing Absorbance with corresponding wavelength for Fuller's earth

Result: The SPF of clay and fuller's earth was found to be 13.326 and 0.814 respectively.

4. Discussion and Conclusion

Skin is the outer covering of the body and it is necessary to protect it from UVB radiations which cause maximum damage, like tanning, sunburns, erythema, wrinkles (photo-ageing).[1]

The market caters to a wide variety of sunscreen products containing different sunscreen agents but some sunscreens

may cause photo-catalytic effect on the skin which is harmful.[2]

Natural materials are being sought as replacements for the synthetic UV protection agents. Potential compounds as natural UV-protection agents in sunscreens include clays and clay minerals, which due to their many benefits for human health are already utilized in various types of pharmaceutical and cosmetic products. [1]

Red soil is easily available in India and it might contain UV protection property. Hence, it was thought that red clay can be evaluated for its Sunscreen capability.

On assessment of physical parameters, the clay was found to be slightly acidic in nature which belongs to Montmorillonite category. It was compared with the fuller's earth which also comes in same category. (Table 4) The acidic and nearly neutral nature of the clay sample may limit the chemical reactions between the skin and clay during application Colour was determined using Munsell Colour chart. (Table 5) The colour of the clay may play a key role in their sunscreen abilities. (20) The colour of the clay used as sunscreen has a hue of 5YR corresponding to the colour of hematite (Fe_2O_3) and geothite. Bulk density may play a key role in the determination of the presence of bulk minerals, moisture content and the texture (Table 6). Texture of the soil was found to be Sandy-Loam (Plate 8). The high content of the moisture in the clay can be a reason for the growth of microbes which make the clay non-usable for cosmetic purpose but it may be reduced by proper sterilization techniques. (Table 7)

From the results obtained of SEM, it was found that particle size was in range of 1.28µm to 1.73µm(Plate 9). From the literature it was found that the smaller the particle size better is the absorption or scattering of radiant energy. This reduces the intensity of UV radiation that reaches the skin [1]. Red Clay can be slightly irritant in high concentration may be due to porous structure (Plate 9).

Microbial evaluation can be used as a basis to see contamination in clay or soil. Red soils are less fertile due to lack of humus and hence microbial growth is low. (Table 3) Microbial evaluation has proved that clay showed negligible growth as compared to fuller's earth which was taken as comparison.

Using XRD measurements (Graph 1), the characterization of natural clay materials using XRD indicated the presence of hematite (Fe_2O_3), Geothite and quartz as the main mineral found in the sample. (Table 9)

The high value of UV absorbance might be associated with the high Fe contents of clay. The magnitude of shift towards high absorbance in the graph (Graph 2) seems to be related to the extent of the higher Fe_2O_3 content. When compared to the absorbance of TiO_2, it has its highest peak at 300nm (21) whereas the absorbance peak of clay sample is 305 nm (Graph 2)

From the above results, it can be concluded that the red soil can be used as effective UV protective agent.

5. Future Scope of Study

Study can be extended to:

1. Incorporation of natural red clay in suitable product and its stability parameters can be carried out.
2. Patch testing on the human volunteers can be carried out and SPF can also be determined.

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