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PHARMACOGNOSTICAL EVALUATION OF MUD THERAPY TRADITIONALLY USED IN PONDICHERRY UNION TERRITORY

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ABSTRACT

Mud is supposed to be one of the best remedies for ailments and thus used as a means of alternative medicines. It is also used in cosmetology as it contains neutral moistures. The use of earth for remedial purposes was very common in ancient times as well medieval ages. In modern times, the mud therapy again came into practice as a valuable therapeutic agent. *Emanuel Felke* a German therapist tried to restore the mud therapy and was nicknamed as clay pastor. *Adolph* just was one of the pioneers of natural cure believed that all disease will be cured if sleeping and laying on earth at night can be made custom. The use of mud therapy has its own socio-economic features in the modern medicines especially in Herpes zoster viral infections, chicken pox, acne, sunburn and various major skin problems. But there is no much scientific evidence still develops for this ancient and currently used treatment. In this current investigation an attempt was made to explore the macro and micro nutrients, elemental composition and pathogenic and non-pathogenic microbial load were evaluated. So this kind of research may have and yield better path to develop mud therapy scientifically as one of the best treatment for various skin ailments in the future world.

Key Words: Mud Therapy, Pharmacognostic evaluation, Atomic Absorption Spectroscopy.

INTRODUCTION

Mud is one of five elements of nature having immense impact on the body in health as well as disease. Mud has a remarkable effect to refresh, invigorate and vitalize the human body. Mud procured for treatment purpose is free from pollution and contamination and is cleaned and sifted before use. Mud therapy includes Mud bath, Mud pack etc [1].

Mud therapy is usually based on two principal elements Prithvi (earth) and Vayu (air). Since mud therapy is used for ancient times for treating health problems however, it is found that number of people still shudder at the thought of applying mud paste on their body. For example one may have seen number of animals rolling about in the mud to get rid of external parasitic infestations on the other hand very few human are willing to experiment with the same. This is because mud is strongly associated with dirt and filth, worms and bugs [2].

It has been found that walking barefooted on dry mud helps in stimulating the acupressure points of soles and consequently, boost the body deficiencies. On the other hand it has been found that sleeping on mud help in reloading our energy levels and literally brings down to earth, so that our body and mind heal through the positive vibrations and magnetic forces which earth have's. It will be surprised to know that a strange form of treatment which is usually done in India, is patient covered in the mud to up the neck for few hours till the person heals. This type of treatment is also known as mud bath. Therefore care is taken to ensure that this mud is free of impurities and animal excreta. Warm water is added into mud and then mud paste is made. After this paste is ready then this paste is applied on individual's body. It will be amazed to see how this much can help us in getting rid of conditions such as psoriasis, eczema and vitiligo [3].

On the above light of information the present study aimed to evaluate the soil sample of Puducherry UT, traditionally used for mud therapy on its physical, chemical and biological aspects, So as to validate the traditional

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treatment scientifically by using modern, sophisticated scientific instrument.

MATERIALS AND METHODS

Preparation of Soil Samples for Analysis

1. The soil samples received at the laboratory is air dried in shade and spread on a sheet of paper after breaking large lumps if present, with a wooden mallet. When air dry, it is further ground by pounding with a wooden mallet in such a way that the aggregate particles are broken down to ultimate soil particles.

2. The soil thus prepared is sieved through a sieve with round holes, 2 mm in diameter. The material on the sieve is again ground and sieved till all aggregate particles are fine enough to pass through and only stones and organic residues remain on the sieve. Mix well the "fine soil" got by sieving and store in a suitable bag with one label on the outside and one inside the container.

Authentication of soil samples

The collected soil samples were authentified by Dr. K. Karunakaran, Agricultural Officer, Agricultural Chemistry Laboratory, Puducherry,

Sub sampling for analysis

The soil in the bag is emptied on a clean thick sheet of paper and evenly spread with a sampling knife. It is heaped into a cone by raising the four end of the paper. It is again mixed well and evenly spread on the paper as before the process is repeated 3 to 4 times to ensure uniformity and then finally spread evenly on the paper again. Now it is divided into four equal quarters and small quantity of soil is taken from various points in each quarters to get a representative sample for the analysis.

ORGANOLEPTIC EVALUATION

The given soil samples were taken and study the following colour, odour, and taste through our sensitory organs and the particle size range was analyzed in microscopic using micrometer.

The soil is rubbed between thumb and fingers before which the soil is moisture (Sand feels gritty, silt feels soapy and clay feels sticky).

Identification of Bacterial Species

Microscopical examination

The soil samples are directly observed by microscopical examination under 10x and 40x objectives for identification of eggs/ova/cyst/larvae of helminthes and protozoans. They were also subjected to different staining methods such Grams staining (for gram(+ve) and gram(-ve bacteria), Albert's staining (for Corynebacterium), Spore staining(for Clostridium), Lactophenol cotton blue (for Fungi) [4].

Culture Method

The direct inoculation procedure involves introducing test samples directly into nutrient media. The *European Pharmacopoeia* (2002) recommends two media: (i) fluid mercaptoacetate medium (also known as fluid thioglycollate medium), which contains glucose and sodium mercaptoacetate (sodium thioglycollate) and is particularly suitable for the cultivation of anaerobic organisms (incubation temperature 30–35°C); and (ii) soyabean casein digest medium (also known as tryptone soya broth), which will support the growth of both aerobic bacteria (incubation temperature30–35°C) and fungi (incubation temperature 20–25°C). Limits are placed upon the ratio of the weight or volume of added sample relative to the volume of culture medium so as to avoid reducing the nutrient properties of the medium or creating unfavourably high osmotic pressures within it. The other media used for the present study are Nutrient agar, Blood agar and Brain heart infusion broth (ready made dehydrated media from Hi media laboratories, Mumbai) were purchased and the media were prepared after autoclaving [5].

Inoculation

1 mg of soil sample was spread all over the media using sterile swab. It is kept under both aerobic incubation and anaerobic incubation at 35^0C for 3 days. Colonies formed were identified by using suitable staining methods mentioned above and biochemical tests.

Quantitative Analysis & Identification of Macro and Micro Nutrients by Atomic-Absorption Spectroscopy (AAS)

- **Preparation of sample solution**

Take 10 gm of soil and add 20 ml of DTPA extraction solution, shake well for 2 hours by using sonicator. Filter it using Whatmann filter paper No.42 (90mm diameter) and the filtrate is subjected to atomizer of atomic absorption spectrophotometer. To Estimate the unknown concentration of zinc, copper, ferric, manganese by using standard curve.

- **Standard curve for Manganese, Iron, copper, and zinc**

Standard solution of manganese and iron of 1000 ppm is diluted into 10, 20, 30, 40, 50 µg/ml and the standard curve was plotted. Standard solution of copper 1000 ppm is diluted to 2, 4, 6, 8, 10 µg/ml and the standard curve was plotted. Standard solution of zinc 1000 ppm is diluted to 1, 2, 3, 4, 5 µg/ml [6].

Determination of Potassium and Sodium by Flame Photometry

- **Preparation of standard curve**

Na, K, and Li solutions that are 1, 5, 10, 20 ,30, 40 ,50 ,60, 70, 80, 90, and 100 ppm of each metal ion dilution were

prepared by using respective standard solution available in the market [7].

- **Preparation of soil sample for analysis**

5 grams of soil is shaken with 25ml of neutral normal ammonium acetate (pH7.0) for five minutes and the contents filtered through a dry filter paper. First few ml of the filtrate may be rejected. Potassium and Calcium is estimated in the extract with the help of flame photometer [8].

Procedure

Adjust the signal, using the Na filter, to zero using distilled deionized water. Read the signal for the Na set of standards and then that of the unknown sample. If the signal obtained for the sample is out of range, dilute a portion of the sample properly till a signal within the range is obtained [9].

Construct a calibration curve for Na in the sample and report the results in ppm.

Estimation of Available Nitrogen in Soil By Alkaline $KMnO_4$ Method

Weigh 20 g of soil and transfer to a kjeldhal flask. Add 20ml of distilled water and 1ml of liquid paraffin or 1g of paraffin wax (to control frothing). Put a few glass beads (with holes) to prevent bumping and then add 100ml of 0.32% $KMnO_4$ solution and 100ml of 2.5% NaOH solution. Distil the contents at a steady rate collecting the liberated ammonia in a 250ml conical flask containing 20ml of boric acid with double (mixed) indicator. Continue the distillation for about 30minutes or until 100ml of distillate is collected in the flask titrate the ammonia collected against the standard acid (N/50) and from the titre value calculate available nitrogen content of the soil.

Determination of Soil P^H

Weigh 20 gm of soil and transfer to 100 ml beaker. All 40 ml distilled water. Stir it well with glass rod and allow it to stand for 30minutes with intermittent stirring. Adjust the pH meter with the buffer solution, wash the electrodes with a jet of water and carefully wipe dry with a piece of filter paper. Immerse the electrodes in the beaker containing soil-water suspension and read the units.

Rating of the soil with reference to pH:

Rating	pH
Acidic	<6.0
Normal	6.1 to 8.0
Tending to become alkaline	8.1 to 8.5
Alkaline	< 8.6

Determination of Soil Electrical Conductivity

Weigh 20 g of soil and transfer to a 100ml beaker. Add 40ml of water, stir it well and allow to stand for half an hour (alternatively use the soil water suspension prepared in pH determination) Switch on the conductivity bridge. Check the instrument with saturated $CaSO_4$ solution and or 0.01 N KCl solution (E.C.2.2m.mhos/cm and 1.41 m.mhos/cm respectively) before proceeding with the sample.

Wash the electrodes with distilled water. Immerse them into the soil suspension. Set the multiplier switch at an intermediate position and rotate the main dial control, until the magic eye of the null indicatory is at its widest. If no shadow angle is obtained or if a shadow angle is only on either end of the scale, set the multiplier switch at another position and repeat. The readings of the scale at this position multiplied by the multiplier switch position indicate the electrical conductivity. Multiply this by the cell constant noted on the cell itself to get specific conductivity.

Rating	Electrical Conductivity
Harmless	0.0 to 1.0
Injurious	1.0 to 3.0
Critical	3 and Above

Determination of Lime Status of Soil

Use the soil water suspension used for pH and E-C. The suspension is allowed for 30 minutes to settle and the water portion is discarded and remaining soil portion is taken for limestone test by using 10% HCl.

10% HCl is taken in a squeeze bottle and tested to every sample by squeezing few ml of 10 % HCl. The presence of effervescence shows the lime content. If it is copious the lime content is more. If the calcium carbonate is more in this test. Detailed estimation of lime status of soil is done. The soil samples are neutralized with excess of standard acid and the excess acid is determined by back titration with standard alkali using phenolphthalein as indicator [10].

RESULTS AND DISCUSSION

Organoleptic Evaluation of Soil Samples

The soil colour was red colour, the odour was sandy odour, taste was sour, the particle size range between 2.66-10.64µm and the texture was found to be sandy soil.

Qualitative Analysis of Bacterial Species

- On Microscopical examination it was identified the collected soil sample have No cyst/eggs/larvae of helminthes.
- In Culture method:

By using different culture method, the sample was analyzed and it was confirmed the sample does not possess any pathogenic bacteria. It only posses non-pathogenic micro-organism such as *Pseudomonas fluorescens*, Arthobacter, Achrombacter, Enterobacter, Micrococcus, *Bacillus subtilis*.

Quantitative Analysis of Macro and Micro Nutrients

By using various modern equipment such as Atomic absorption spectroscopy, Flame photometry, Kjeldal flask, the various macro and micro nutrients were estimated in the soil sample and the results were placed in the table 1.

Table 1. Quantitative Analysis of Macro and Micro Nutrients

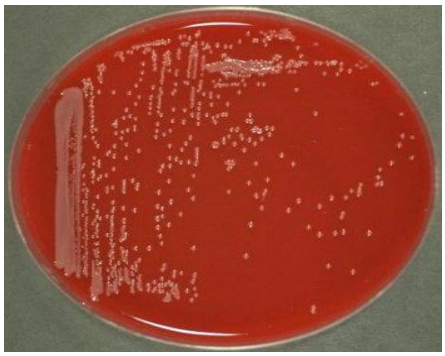
S.No	COMPONENTS	Amount present	
		Soil Sample-1	Soil Sample-2
1	Potassium Oxide(K_2O)	887 kilo/acre	65 kilo/acre
2	Manganese (Mn)	2.651 ppm	1.878ppm
3	Zinc(Zn)	0.4786 ppm	2.052ppm
4	Iron (Fe)	2.893 ppm	3.056 ppm
5	Copper (Cu)	0.265 ppm	0.306 ppm
6	Phosphorus Oxide (P_2O)	0.26 kilo/acre	0.36 kilo/acre
7	Nitrogen (N)	77.50 kilo/acre	137.50 kilo/acre

MISCELLANEOUS

Table 2. P^H, Electrical conductivity, Lime status were state below

S.NO	PARAMETER	RATIO
1.	Soil pH	5.7(acidic)
2.	Electrical Conductivity	3.5(critical)
3.	Lime Status	Normal

Fig 1. Bacterial Species Identified

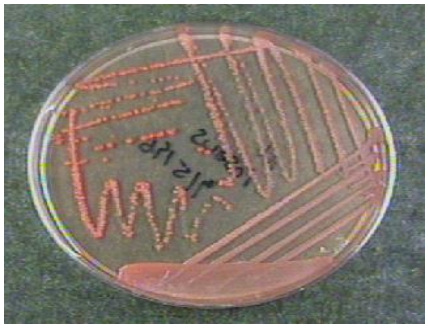


Pseudomonas fluorescens

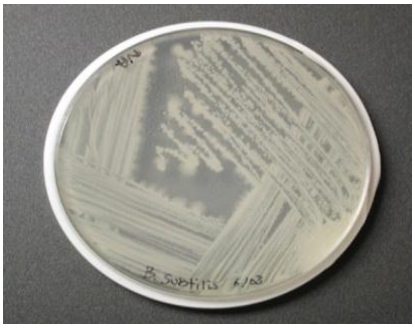
Arthobacter

Achrombacter

Enterobacter



Micrococcus



Bacillus subtilis

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