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An *in-vitro* study on antifungal activity of *sepiofficinalis*, *tellurium metallicum* and their potencies against *Trichophyton rubrum*

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Abstract

In this research, we evaluated the growth-inhibiting effects of *sepiofficinalis* and *tellurium metallicum*, along with their strengths against *Trichophyton rubrum*. The objective of the current study is to examine the drug's influence. Homeopaths have been attempting to validate the remarkable efficacy of minute doses for nearly decades. The overarching implication of this research is to demonstrate the effect of the chosen homeopathic medicine on *Trichophyton rubrum*. An inoculum of the fungal strain *Trichophyton rubrum* was suspended in 2ml of MHA broth and incubated at 37°C for one day to achieve a turbidity of 0.5 McFarland standards. The antifungal efficacy was assessed using the disc diffusion method. For this procedure, the inoculum was spread over Muller Hinton Agar medium with a sterile swab. Small circular sterile paper discs (6mm in diameter) were impregnated in the MHA plates, and a known quantity of 30C, 200C, and 1M of *sepiofficinalis* and *tellurium metallicum* was applied to the discs using a micropipette. The plates were left at room temperature to allow sample absorption into the medium and were incubated at room temperature for 24 to 48 hours. The antifungal activity was determined by measuring the diameter of the inhibition zone. Fluconazole served as the positive control, while ethanol was the negative control.

In this study, *Sepia officinalis* 30C, 200C, 1M and *Tellurium metallicum* 30C, 200C, 1M did not exhibit any inhibitory activity against *Trichophyton rubrum* fungi. This experiment clearly indicated that the dilutions of both *Sepia officinalis* and *Tellurium metallicum* did not produce any inhibition zone.

Keywords: Kirby bauer method, mueller hinton agar, *Trichophyton rubrum*, *Sepia officinalis*, *Tellurium metallicum*

Introduction

Microorganisms that fall under the eukaryotic classification include fungi. Types of fungi can encompass molds, yeasts, or a combination of both. Certain fungi can lead to various illnesses, including allergic, systemic, superficial, cutaneous, or subcutaneous conditions. Yeasts are small fungi composed of single cells that reproduce through budding ^[1]. In contrast, molds develop as apical extensions of long filaments known as hyphae. Hyphae can contain a varying number of nuclei and may be either regularly or sparsely septate. The digestion of food by fungi occurs externally, as they release hydrolytic enzymes into their surrounding environment ^[2]. This method of nutrition is referred to as absorptive nutrition and takes place irrespective of the fungi's form or size. Worldwide, *Trichophyton rubrum*, commonly referred to as *T. rubrum*, is the dermatophyte responsible for the majority of superficial fungal infections ^[3]. Dermatophytes, a subclass of fungi, have the ability to invade keratinized tissues such as skin, hair, and nails. While this type of fungus can infect any area of the skin, it most commonly affects the feet, scalp, axillae, inguinal region, and nails ^[4].

Parasitic fungi face challenges in surviving on humans. Four criteria must be fulfilled ^[5].

- 1) It must be able to grow at temperatures of 37°C or higher, which is considered a high temperature.
- 2) The parasite must penetrate the tissues it intends to infect, either by breaching the host tissue barriers or by evading them with tiny airborne cells that can enter the sinuses and lungs, which are air-filled regions.
- 3) It must possess the ability to absorb and digest components of human tissues.
- 4) Finally, it should be resistant to the human immune system.

The dermatophyte most commonly associated with athlete's foot is *T. rubrum*. While other dermatophytes can also cause this condition, they are less frequently isolated from humans. In locker rooms and public showers, *T. rubrum* spores can survive on human scales for up to a year, making them easily transmissible from one person to another^[6].

Homeopathy serves as a versatile therapy, delivering affordable solutions for numerous illnesses. Extensive research with scientific validation has proven the effectiveness of homeopathic treatments in managing different health issues.

Sepia officinalis is a homeopathic remedy, this remedy is prepared from the ink of the common cuttlefish. In homeopathy it is suggested for certain chronic skin eruptions, itching, ringworm-like patches, or hormonal-linked skin changes. Some indications are Vesicular eruptions in the armpits, on the tips of the elbows; eruptions that accumulate into large crusts on the elbows; thick crusts develop on the joints; eruptions between the fingers; moist eruptions that exude a watery fluid or thick, yellow, purulent discharge^[7].

Tellurium, a metal, is the origin of the medicine known as Tellurium Metallicum. When in its raw state, this metal remains inert. The homeopathic remedy Tellurium Metallicum is developed through potentization. It is particularly useful in addressing ailments like ringworm, otitis media, ear discharges, and sciatica, among various other conditions. Moreover, it is recommended for treating ringworm lesions on the face and lower extremities that may have deteriorated over time. The skin surrounding these ring-shaped lesions tends to feel quite warm. This treatment is also particularly useful for managing eczema, which appears as circular patches, small red bumps, and tiny fluid-filled blisters. Its use is strongly suggested for eczema that occurs behind the ears, where a thick crust may develop^[8].

Materials and Methodology

Study design: The antifungal property of Homoeopathic dilutions against a particular strain of *Trichophyton rubrum*.

Study setting: Sarada Krishna Homoeopathic Medical College Research Lab, Kulasekharam.

Drug for the study: Medicines were procured from Homoeopathic pharmaceuticals. In this study, Homoeopathic medicine *sepia officinalis* 30C, 200C, 1M and tellurium metallicum 30C, 200C and 1M was used.

Type of the study: An experimental *in vitro* study.

Fungal strain: Standard strains of *Trichophyton rubrum* were used for this study. The culture was grown in nutrient agar media.

Antifungal screening: In this study, a Muller Hinton agar plate was used for antifungal screening.

Preparation of Disc: Plain sterile disc was purchased from Hi media and soaked with each concentration of extracts and placed at room temperature to get air dry for 6 (six) hrs. Then, the disc paper was labelled and used for antifungal study.

Method of Collection of Data: The drugs used for conducting the study are *sepia officinalis* 30C, 200C, 1M

and tellurium 30C, 200C, 1M. One negative control is also kept, which is ethanol. One positive control, which is fluconazole. Thus, five groups are formed which are shown in the following table.

Table 1: Samples and Groups

GROUP I	
I	Negative control - ethanol
II	Positive control - fluconazole
III	<i>Sepia officinalis</i> 30C
IV	<i>Sepia officinalis</i> 200C
V	<i>Sepia officinalis</i> 1M
GROUP II	
I	Negative control - ethanol
II	Positive control - fluconazole
III	Tellurium metallicum 30C
IV	Tellurium metallicum 200C
V	Tellurium metallicum 1M

Mueller hinton agar

Currently, MHA is predominantly utilized for the routine susceptibility testing of nonfastidious microorganisms using the Kirby-Bauer disk diffusion method. The agar was prepared by dissolving 38 g of the medium in one liter of distilled water. This mixture was then heated with constant agitation and boiled for one minute to ensure complete dissolution of the medium. Following this, it was autoclaved at 121°C for 15 minutes and allowed to cool to room temperature^[9]. The cooled solution was subsequently poured into sterile petri dishes placed on a flat, horizontal surface to achieve a uniform depth. The final pH was then verified. The plates were stored at 2-8 degrees Celsius. After incubation, the inhibition zones were assessed around the disc. The diameter of the zone of inhibition (including the disc) was measured in millimeters (mm). The lack of an inhibition zone was interpreted as a lack of activity. The results were categorized as resistant if the zone of inhibition was less than 7 mm, intermediate if it was between 8-10 mm, and sensitive if it exceeded 11 mm^[10].

Kirby-Bauer method

The antifungal activity was conducted using the Kirby-Bauer method. The Muller Hinton Agar plate was prepared and allowed to solidify. Once the plates had solidified, the *Trichophyton rubrum* inoculum was evenly swabbed across the surface of the Muller Hinton plate. Sterile forceps were then used to place 6 mm sterile discs in the designated positions^[11]. Ethanol served as the negative control, while fluconazole acted as the positive control. After a few minutes, various potencies {30C, 200C, and 1M} of the homeopathic medicines *sepia* and tellurium were introduced into the sterile discs under sterile conditions. Each sterile disc received 10 µl of the medicine. The plate was left undisturbed and subsequently incubated at 37°C for 24 hours. After this incubation period, the results were assessed by measuring the zone of inhibition in millimeters^[12].

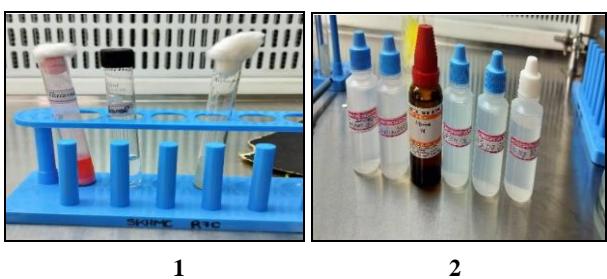
Observation and Result

In vitro antifungal study was done against *Trichophyton rubrum* with *sepia officinalis* and tellurium metallicum, in its different potencies such as 30C, 200C, 1M. Ethanol was used as negative control. Fluconazole was used as positive control. The antifungal activity was performed following Kirby-Bauer method. In the plates the discs were denoted as shown in the following table.

Table 2: Code and their Samples

Code	Samples
S30	Sepia officinalis 30C
S200	Sepia officinalis 200C
S1M	Sepia officinalis 1M
E	Negative control - Ethanol
P	Positive control - Fluconazole
T30	Tellurium 30C
T200	Tellurium 200C
T1M	Tellurium 1M
E	Negative control - Ethanol
P	Positive control - Fluconazole

The plate was incubated at 37°C for 24 hours. After 24 hours the results were observed by measuring the zone of incubation in millimeters. Here, the selected homoeopathic medicine doesn't showed any inhibitory action against *Trichophyton rubrum*.



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Picture 1 and 2: Medicines, Ethanol, Fluconazole and *T. rubrum* Culture



Picture 3: Swabbing of suspension



Picture 4: Placing Discs and Adding Medicines



Picture 5: After 24 hours - zone of inhibition

Table 3: Sample and their Zone of inhibition

Sample	Zone of inhibition
S30	0.2 mm
S200	0.2 mm
S1M	0.2 mm
E	0.2 mm
P	0.4 mm
T30	0.3 mm
T200	0.2 mm
T1M	0.2 mm
E	0.2 mm
P	0.5 mm

Discussion

The primary cause of the disease is always the derangement of vital force by any poisonous miasmatic forces, which is followed by functional and structural changes. Thus, pathology is the result of the vital force's derangement by noxious miasmatic forces, and symptoms are the result of the vital force's suffering. In short, homoeopathic medical science views the facts of the universe in general and the facts of medicine in particular from a vitalistic - substantialistic standpoint because functional changes always precede structural changes [13].

"The fact that this extremely ancient infecting agent's psora, as stated in Aphorism 81 by Hahnemann, contribute to the production of these greater varieties of chronic diseases nothing but the secondary manifestation of the psora which has hitherto been treated of in old pathological works under a number of special names as disease of an independent character" [14].

By the above statement it is very clear that pathological symptoms are the expressions of disease and then are the effects of miasmatic causes. Hence various pathological names of various diseases caused by various bacteria and fungi are primarily the results of deranged vital force by the miasmatic forces especially psora.

In the above conducted *in vitro* experiment, Sepia officinalis and Tellurium did not show inhibitory activity against the fungus. This proves that homoeopathy theory of single medicine treats whole body.

As per Stuart Close, the totality of symptoms can be considered as the basis of a homoeopathic prescription, represents the therapeutic idea. This is applicable in pathology also. Disease in general, is considered as a whole is composed of totality of all the symptoms which represent it to our senses. The pathological totality, can be personified or pictured by the imagination in the form of a human being. However, identification of active compounds in the homoeopathic formulations and how homoeopathic medicines work to inhibit the growth of human pathogenic fungi *Trichophyton rubrum* *in vitro* has not been evaluated in this study which is the subject of further investigations.

Conclusion

From this study, it is shown that Sepia officinalis and Tellurium metallicum do not exhibit any *in-vitro* antifungal activity against *Trichophyton rubrum*. Based on the methodology used, the recorded zones of inhibition (0.2 mm to 0.3 mm) fall well below the 7 mm threshold, confirming that the fungus is resistant to these medicines in a laboratory setting.

However, it is argued that there is no chance of developing drug resistance to homeopathic remedies, as they act dynamically on the vital force rather than through direct chemical suppression. Hence, it is crucial to acknowledge the scope of Sepia officinalis and Tellurium in the homeopathic treatment of *Trichophyton rubrum* infections. To establish their clinical efficacy beyond the laboratory

model, rigorous clinical trials and systematic studies are required.

Conflict of Interest

There are no conflicts of interest related to this study.

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