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IN VITRO ANTI-MYCOBACTERIAL STUDY OF ESSENTIAL OIL OF FEW SELECTED PLANTS – PART 2

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ABSTRACT

Emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *Mycobacterium tuberculosis* has further complicated the problem of tuberculosis (TB) treatment. It was thus very essential to find out a remedy for this killer disease. In this article which is a preliminary study to show that certain essential oils possess anti-mycobacterial activity. In this study, essential oil of few Ayurvedic plants like Turmeric Oil, Nilgiri Oil and the Oleo Resin part of Kalimari were screened individually and in combinations for their anti-mycobacterial activity and promising results were found against the *Mycobacterium smegmatis*, a non-pathogenic species of *Mycobacterium* group. Further analysis and research is in progress.

KEYWORDS:

Tuberculosis,
Mycobacterium,
Ayurvedic herb,
Essential oils.

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1. INTRODUCTION :

Thioamide drugs, ethionamide (ETH) and prothionamide (PTH) have been widely used for many years in the treatment of mycobacterial infections caused by *Mycobacterium tuberculosis*, *M. leprae*, and *M. avium* complex infections [1-2]. ETH and PTH are both bacteriocidal and are essentially interchangeable in a chemotherapy regimen. They are the most frequently used drugs for the treatment of drug-resistant tuberculosis and, therefore, are becoming increasingly relevant as the number of multi drug resistant and extensively drug-resistant cases is increasing worldwide [3-4].

With the current trend in the biotechnology of plant tissue culture [5], it would appear that man may soon have to depend on plants as a source of a number of antimicrobial agents since these plants will most likely continue to produce antimicrobial agents which could be used against infections by microorganisms. Natural products are proven templates for the development of new scaffolds of drugs [6-8]. They have received considerable attention as potential anti-TB agents [9].

Phytochemical aspects of most medicinal plants have been known and used since time memorial [10-11]. Ethanobotanical advantages conferred by these plant based products have surpassed the chemical counter parts owing to their lesser side effect and more potent therapeutic effect. Natural products continue to play the most significant role in the drug discovery and development process [12]. Hence it is a demanding need of the hour to study the various pharmacologically valuable aspects of these medicinal plants. Therefore, the present study was carried out to check the anti-mycobacterial activity of essential oil of three plants viz. Turmeric Oil [13-16] Nilgiri Oil [17-19] and oleo resin part of Kali Mari [20-23].

MATERIALS AND METHODS:**Preparation of the Test Drug**

The study was designed to be done in three different phases.

Phase 1: To check efficacy of individual drug substance in 1:1 concentration with DMSO

Phase 2: To check efficacy of individual drug substance in 2:1 concentration with DMSO

Phase 3: To check efficacy of drug substances in combination as mentioned in table 1.

Table 1: The various trials and their combinations used in the study

Sample	Oil : DMSO	Oil : DMSO
TO	1:1	2:1
NO	1:1	2:1
KMO	1:1	2:1
TO + NO	1:1:1	2:2:1
TO + KMO	1:1:1	2:2:1
NO + KMO	1:1:1	2:2:1
TO + NO + KMO	1:1:1:1	2:2:2:1

Abbreviations: TO: Turmeric Oil, NO: Nilgiri Oil, KMO: Kali Mari Oleo Resin

Test Organism: *Mycobacterium smegmatis* was used as the test organism. The organism was procured from MTCC Chandigarh, ATCC number 14468. It is a non pathogenic strain with safety level 1 and is considered to be safe for laboratory experiments. The sample was immediately revived on Middlebrook 7H9 Broth base and later transferred onto Lowenstein Jensen Medium.

Preparation of Mc Farland standard Turbidity Standards:

Mc Farland standard 0.5 was prepared by adding specific volume i.e. 1.174% barium chloride into 1% sulphuric acid. Mac Farland 0.5 standard was used in this study, which contains 99.5ml of 1% sulphuric acid and 0.5 ml of 1.174% barium chloride. Standard solution is dispensed into tubes to get comparable to those used for inoculum preparation. These tubes were then sealed tightly and stored in dark at room temperature. The Mc Farland 0.5 standard provides turbidity comparable to a bacterial suspension containing 1.5×10^8 cfu/ml (NCCLS 1993).

In Vitro Anti Bacterial Study: Middlebrook 7H9 agar was used for the study. It was mixed with hot distilled water and glycerol and autoclaved at 15lb pressure for 15 minutes. After autoclaving, it was allowed to cool to 45°C-50°C. Middlebrook ADC growth supplement was then added to enrich the medium and to allow the growth of *Mycobacterium*. The medium was poured into sterilized Petri dishes with a uniform depth of approximately 4 mm. The agar medium was allowed to solidify and then stored at 4°C till used for further analysis. The media was procured from standard Hi Media supplier

The modified agar-well diffusion method was employed to study the antibacterial activity of the plant essential oils. A sterile cotton swab was saturated by dipping into standardized bacterial culture. Lawn culture of the test strain was prepared by swabbing to give a uniform inoculum to the entire surface. The plates were allowed to dry, after which wells were bored with the help of a cork borer and 0.1 ml of the sample was loaded into the well. The test was carried out in duplicates to get a mean value. The plates were then incubated at 37°C for initial 18-24h and then more if required.

RESULTS & DISCUSSION:

The results of the study carried out in three phases are as shown in Table 2. The actual concentration of the actives is calculated as below:

When we take the 1:1 ratio of the samples i.e. 1mL+1mL = 2000µL of this we take 0.1mL of sample for test. When we calculate the effective concentration of sample in 0.1mL of sample it is 50µL of the test oil. In a similar manner when we calculate the effective concentration of sample in 0.1mL of sample for 2:1 ratio it is 66.6µL, for 1:1:1 ratio it is 33.3 µL , for 2:2:1

ratio it is 40µL, for 1:1:1:1 ratio it is 25µL and for 2:2:2:1 ratio it is 28.56µL.

Table 2: The Zone of Inhibition of the samples tested

Sample	Ratio	Concentration per 100µL	Result
TO + DMSO	1:1	50µL: 50µL	09mm
NO+ DMSO	1:1	50µL: 50µL	10mm
KMO+ DMSO	1:1	50µL: 50µL	10mm
TO+ DMSO	2:1	66.6µL: 66.6µL	21mm
NO+ DMSO	2:1	66.6µL: 66.6µL	22mm
KMO+ DMSO	2:1	66.6µL: 66.6µL	22mm
TO + NO+ DMSO	1:1:1	33.3µL: 33.3µL: 33.3µL	18mm
TO + KMO+ DMSO	1:1:1	33.3µL: 33.3µL: 33.3µL	14mm
NO + KMO+ DMSO	1:1:1	33.3µL: 33.3µL: 33.3µL	18mm
TO + NO+ DMSO	2:2:1	40µL: 40µL:20µL	18mm
TO + KMO+ DMSO	2:2:1	40µL: 40µL:20µL	15mm
NO + KMO+ DMSO	2:2:1	40µL: 40µL:20µL	20mm
TO + NO + KMO+ DMSO	1:1:1:1	25µL: 25µL: 25µL: 25µL	14mm
TO + NO + KMO+ DMSO	2:2:2:1	28.56µL: 28.56µL: 28.56µL: 28.56µL	15mm

Abbreviations: TO: Turmeric Oil, NO: Nilgiri Oil, KMO: Kali Mari Oleo Resin

The Zone of Inhibition ranging from 9mm to 22mm is quite a good indication of the inhibition.

The results demonstrate that all the three samples tested showed good anti mycobacterial results when tested in equal ratios and in increased concentrations.

CONCLUSION:

Thus from the above results it can be concluded that all the samples tested have good efficacy and the oleo resin part of Kalimari having slightly better activity.

It can also be observed that the sample in single are showing to possess better activity than those in combination, so a conclusion can be drawn that their effect in single and in increased dosages tend to possess slightly better activity than in combinations with each other.

However this is a preliminary research work and more detailed work on finding anti-TB agent from natural sources is in progress.

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