

# Antitubercular Activity of Essential Oils Extracted from Some Aromatic Plants, Flowers and Seeds

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## ABSTRACT

**Background:** Essential oils are the plant secondary metabolites, which are a complex mixture of flavoring volatile compounds. The biosynthesis of these volatile compounds by the plant is intended for chemical communication. They are largely meant for activities like antimicrobial, cytoprotection, antioxidant and insect attraction to assist pollination. Essential oils largely contain a unique mixture of mono and di terpenes. In the present work, we isolated essential oils from different plant parts including leaves, flowers and fruits which are used in our daily life and evaluated their antitubercular activity and compared with antibacterial and anti-fungal activity. **Materials and Methods:** The isolation of essential oils (As these are ethereal compounds) were carried out by solvent extraction method and evaluated for their antibacterial and antifungal activity by Cup-plate agar diffusion method, and the antitubercular activity was evaluated by MABA method. **Results:** Among the samples tested, the flower extracts of Mari gold (Orange and yellow), *Tagetes patula*, Chrysanthemum (White, Yellow, Maroon and Purple), Roses (Red and orange), the flower petals and stalks of Night jasmine (*Nyctanthes arbor-tristis*) and Jasmine (*J. officinalis* and *J. multiflorum*) shown potent antitubercular and good antibacterial activity. All these flower extracts shown good anti-fungal activity except Jasmine species. Significant anti-fungal action was seen in the extracts of umbelliferous fruits like fennel, cumin, and caraway. Whereas aromatic plants (Mint and Sweet Marjoram) have shown both antibacterial and antifungal activity. **Conclusion:** Essential oil of all the samples has shown potent antitubercular activity when comparing with standard drugs Rifampicin, Streptomycin etc, and it was noted that these are having selectivity towards mycobacterium when comparing with bacteria and fungi.

**Keywords:** Chemical communication, Ethereals, Essential oils, Antitubercular activity.

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## INTRODUCTION

Chemical communication is the process in which one organism (Animal or plant) may communicate with other organism of same or different species through chemical signals, called as semiochemicals. These chemical signals are volatile in nature and produce from outside of body and release into environment. Basically, these are of two types - Pheromones and Allochemicals. Pheromones – involves in communication between members of the same species, Whereas Allochemicals – to communicate different species. Pheromones have the power to alter a recipient's behavior and can be used for mating, alarming, attracting, recognising, and trail-following. Not only that, but some plant pheromones also involved in self-defense.<sup>1-3</sup> Terpenoids are potent molecules meant for chemical communication as pheromones and

produced as a part of defense system to fight with biotic and abiotic stress. Pheromones can contain significant or moderate amounts of essential oil (Mono and Sesqui terpenes) components. These are useful against both pathogenic and non-pathogenic disorders and have pharmacological properties such as anti-inflammatory, antioxidant, antidiabetic, anticarcinogenic, and cardiovascular protective activity.<sup>4,5</sup>

There is need for discovery of novel antitubercular agents to manage drug resistance problem. We used Terpenoids to obtain Mechanism of action to treat *Tuberculosis*, as many of terpenes are known to possess unique Mechanism of action. India is represented by rich culture, traditions and natural biodiversity. In terms of herbal medicines, India is a world leader and providing knowledge to researchers in searching novel drug molecules. In this regard we selected some Flowers, aromatic Plants and umbelliferous seeds containing volatile oils to act against *Tuberculosis*.<sup>6,7</sup> In the present work, we collected essential oils from flowers which are used in pooja like Mari gold (Orange and yellow), French marigold *Chrysanthemum* (White, Yellow, Maroon and Purple) and Jasmine (*J. officinalis*



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and *J. multifloram*), Roses (Pink, red and orange), Night jasmine (*parijata*), umbelliferous fruits like Fennel, Cumin, Caraway and aromatic plants like Mint, Coriander and Sweet Marjoram, tested for their activity against bacteria, fungal and Tuberculosis and reported it.

## MATERIALS AND METHODS

### Collection of flowers and extraction

All the flowers, aromatic plants and umbelliferous seeds are collected freshly from surroundings of Guntur, A.P., India. The collected material was cleaned and allowed for maceration in Diethyl ether for 2 days at 20°C. The extract was collected by squeezing the macerated material. The solvent was removed by drying at below 20°C and properly stored till further use.

### Antibacterial activity

The antibacterial activity was evaluated by Cup-plate agar diffusion method. The petridish with solidified medium was divided in to 3 portions and with the help of borer made the bores having diameter 8mm. 10 $\mu$ l of test and standard (Streptomycin) having the conc. 100  $\mu$ g/mL were poured into bores. To get uniform distribution of drugs, plates were allowed to stand for 2hr by keeping them into refrigerator, after that allow these plates for incubation for one day at 37°C. Zone of inhibition was measured.

### Antifungal activity

Antifungal activity was evaluated by Cup-plate agar diffusion method, by following same procedure by using the *Aspergillus* as organism and Ketoconazole is the standard. All the Petri plates were incubated for 48hr at 37°C and Zone of inhibition was measured.

### Antitubercular activity using Microplate Alamar Blue assay (MABA) method

Using a Microplate Alamar Blue test, compounds' anti-mycobacterial activity was evaluated against *M. tuberculosis* (H37 RV strain) (MABA). This method has good agreement with the proportional and BACTEC radiometric methods, is non-toxic, and makes use of a thermally stable reagent. Into a sterile 96 wells plate 0.2ml of sterile deionized water, 100  $\mu$ l of the Middle brook 7H9 broth was taken. To this mixture a serial dilutions of test compounds having the concentration range from 0.2 to 100  $\mu$ g/ml were added. The plate was sealed with parafilm and incubated for 5 days at 37°C. A mixture (1:1) of 10% Tween 80 and Almar Blue reagent was added and again incubate it for 24 hrs. Color change from pink to blue is the measure of activity and MIC is the parameter. The anti T. B activity of sample essential oils and the standards are represented in Figures 1, 2 and 3 respectively.

## RESULTS

In the present work we evaluated the antibacterial, antifungal and antitubercular activity by *in vitro* method, which are safe and less expensive. The results were represented in Table 1. For antibacterial and antifungal activities zone of inhibitions were calculated and for antitubercular activity MIC'S were represented.

## DISCUSSION

The duration and complexity in treatment leads to non-adherence, Adverse events, XDR and MDR, Co-infection of T.B with HIV, Identification and treatment of Latent TB persons, DOTS program which is labor-intensive and expensive are factors making the treatment and complete elimination of Tuberculosis very difficult. We got an opportunity to do research on plant products which are used in the treatment of Tuberculosis.<sup>6</sup> The Aromatic plants belonging to Amarylidaceae, Apiaceae, Asparagaceae, Asteraceae, Leguminosae, Rutaceae and Solanaceae are having antitubercular activity because of the presence of essential oils.

The samples of essential oils from flowers, seeds and aromatic plants have shown good results. The essential oils of flowers shown good antibacterial activity, particularly *Chrysanthemum morifolium* (White) have shown potent activity, whose zone of inhibition is 20mm when comparing with standard. These essential oils have shown less effect on fungi except oils of *Nycanthus*, whose zone of inhibition was 18mm, which is more than that of standard (15mm). The essential oils of aromatic plants shown good antibacterial and antifungal activity, when comparing with standard these are less significant. The essential

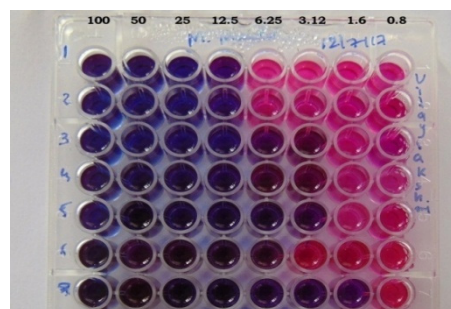


Figure 1: Anti-tubercular activity of essential oils.

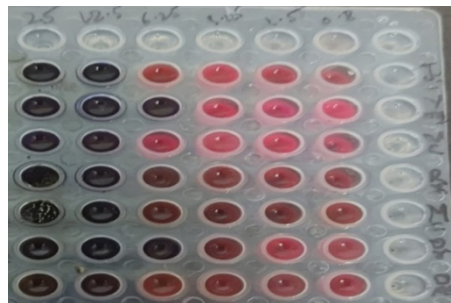
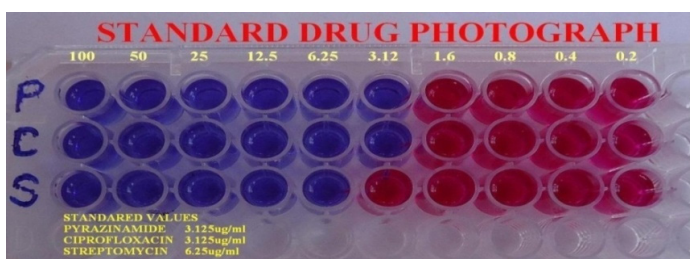


Figure 2: Anti-tubercular activity of essential oils.

**Table 1: Antitubercular, Antibacterial and Antifungal activities of essential oils of flower, aromatic plants and umbelliferous seeds.**

| Sl. No                     | Sample (100 µg/ml) | Antibacterial activity   | Antifungal activity      | Antitubercular activity          |
|----------------------------|--------------------|--------------------------|--------------------------|----------------------------------|
|                            |                    | Zone of inhibition (cms) | Zone of inhibition (cms) | MIC (Conc. in µg/ml)             |
| 1                          | MGY                | 1.4                      | 0.9                      | 12.5                             |
| 2                          | MGO                | 1.2                      | 1.4                      | 12.5                             |
| 3                          | KBY                | 1.6                      | 1.6                      | 6.25                             |
| 4                          | CYY                | 1.8                      | 0.9                      | 12.5                             |
| 5                          | CYW                | 2.2                      | 1                        | 6.25                             |
| 6                          | CYM                | 1.7                      | 0.9                      | 6.25                             |
| 7                          | CYP                | 1.3                      | 0.9                      | 3.12                             |
| 8                          | JAO                | 1.2                      | 1.2                      | 6.25                             |
| 9                          | JAM                | 1.5                      | 1.5                      | 6.25                             |
| 10                         | NFP                | 1.2                      | 1                        | 3.12                             |
| 11                         | NFS                | 1.8                      | 1.8                      | 1.6                              |
| 12                         | R. rose            | 1.2                      | 1.1                      | 12.5                             |
| 13                         | O. rose            | 1.4                      | 1.8                      | 6.25                             |
| 14                         | P. rose            | 1.5                      | 0.9                      | 6.25                             |
| <b>Aromatic plants</b>     |                    |                          |                          |                                  |
| 15                         | OMM                | 1.7                      | 1.2                      | 12.5                             |
| 16                         | CSP                | 1.2                      | 0.9                      | 12.5                             |
| 17                         | MAP                | 1.6                      | 1.6                      | 12.5                             |
| <b>Umbelliferous seeds</b> |                    |                          |                          |                                  |
| 18                         | CSS                | 1.0                      | 0.9                      | 6.25                             |
| 19                         | CCZ                | 1.1                      | 1.4                      | 12.5                             |
| 20                         | FVF                | 1.0                      | 2.4                      | 3.12                             |
| 21                         | CCL                | 1.0                      | 1.2                      | 6.25                             |
| 22                         | Standard           | 2.4 (Streptomycin)       | 1.5 (Ketoconazole)       | Pyr- 3.12, Cip-3.12<br>Str-3.12. |

**Figure 3:** Anti-tubercular activity of stand. drugs like Pyrizinamide, Ciprofloxacin and Streptomycin.

oils of Umbelliferous seeds have shown good antibacterial and antifungal activity, when comparing with standard these is less significant. Coming to the antitubercular activity, the essential oils of flowers showed good antitubercular activity, than that of aromatic plants and seeds. Particularly oil from *Nycanthus* is more potent than the standards like Pyrizinamide, Ciprofloxacin and streptomycin, whose MIC is 1.6µg/mL. Oil from *Chrysanthemum*

*morifolium* (Maroon) have shown activity equal to that of standard i.e., 3.25µg/mL. Remaining other flower oils showed their activity at 6.25µg/mL. The essential oils of aromatic plants showed their activities at 12.5µg/mL, when comparing with standard these are less significant. The essential oils of Umbelliferous seeds showed good activity and the MIC of fennel was 3.125µg/mL which is equal to that of standard. MIC of Coriander and Clove was 6.25µg/mL and MIC of Zeera was 12.5µg/mL, when comparing with standard these is less significant.

So, when comparing the antifungal and antibacterial activities of these essential oils, they shown more specificity towards *Mycobacterium tuberculosis*. This was proven by the Concentration of samples used for the evaluation, 100µg/mL in case of both antifungal and antibacterial activities and where as MIC-1.6 µg/ml for antitubercular activity, which is less than that of standard.

## CONCLUSION

From this study we are concluding that the essential oils very unique in nature and has several uses in our day-to-day life. The selected flowers in using as a part our tradition (like festivals, as ornamental and decorative) and umbelliferous seeds and aromatic plants like Mint using in daily life are having potent antitubercular activity and playing an important role in maintaining our environment hygiene and making us healthy.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**MIC:** Minimum inhibitory concentration; **µg/mL:** Microgram per Milliliter; **µl:** Micro liter.

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