



ANTIMICROBIAL EFFECT OF PLECTRANTHUS AMBOINICUS AND OCIMUM TENUIFLORUM AGAINST MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Antimicrobial agents are the chemical substances which kill microbes or prevent the growth of microbes. Infectious diseases are caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multicellular parasites. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, hence an alternative therapy is needed. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality. In this study Aqueous and alcoholic extracts of two medicinal plants are subjected to in-vitro

antibacterial assay against human pathogenic mycobacterium employing cup diffusion method. Among two plants tested Ethanolic extracts of Ocimum tenuiflorum and Plectranthus amboinicus the most effective against MTB. Other than methanolic leaf extract was less effective against MTB. Largest zone of zone of inhibition of 7mm was obtained with Ethanolic extract of Ocimum Tenuiflorum and Plectranthus Amboinicus plants against MTB, Methanolic extract zone of inhibition of 5mm was obtained with Methanolic extract of Ocimum Tenuiflorum and Plectranthus Amboinicus.

KEYWORDS: Bacteria, Ocimum tenuiflorum, Plectranthus amboinicus, Zone of inhibition.

INTRODUCTION

Plants have been the basis of different traditional medicinal systems throughout the world and

continue to provide mankind with new remedies. World health organization defines traditional medicines as therapeutic practices that have been in existence often for hundreds of years before the development and spread modern medicines and are still in use today.^[1] Medicinal plants play a key role in the development of potent therapeutic agents. plant based drugs excellent contribution to modern therapeutics. Plant derieved drugs are used to cure mental illness, skin disesase, tuberculosis, diabetes, jaundice, hypertension and cancer. The use of plant material used as indegenous cure in traditional system of medicine.^[2-4] A wide range of medicinal plant part is used for extract as raw drugs and they possess varied medicinal properties.^[5]

An antimicrobial is an agent that kills microorganism or inhibits their growth.^[6] Tuberculosis is an infectious disease caused by the bacterium mycobacterium tuberculosis. Tuberculosis generally affects the lungs but can also affect other parts of the body. Most infections do not have symptoms, in which case it is known as latent tuberculosis. When compared with other diseases caused by a single infectious agent. TB is a second biggest killer, globally.^[7] TB is more common in developing countries about 80% of the population in many Asian and African countries test positive in tuberculin test, while only 5-10% of the US population test positive.^[8] TB medication to be toxic to the liver and although side effects are uncommon when they do occur, they can be quite serious potential side effects like dark urine, fever, jaundices, loss appetite, nausea and vomiting.^[9]

In the present study, the crude leaf extracts of *Ocimum Tenuiflorum* with two solvents ethanol and methanol were used to monitor antibacterial property against *M. segmatis*.^[10] *Ocimum Tenuiflorum* also known as *Ocimum Sanctum*, Holy basil, or Tulasi, is an aromatic plant in the family Labiatae, leaves are green or purple they are simple petiole with an ovate up to 5cm long blade which usually slightly purplish flowers are placed in closed whorls on elongate racemes.^[11-12] It is important to pinch tops of tulasi plants when they are forming four or six pairs of leaves, this will make the plant grow bushier.^[13-14]

In the present study, the crude leaf extracts of *Plectranthus Amboinicus* with two solvents ethanol and methanol were used to monitor antibacterial property against *M. segmatis*. *Plectranthus Amboinicus*, once identified as *Coleus Amboinicus*, is fleshy perennial plant in the family Lamiaceae with oregano like flavor and odour, native to Southern and Eastern Africa from South Africa.^[15]

It is a large highly aromatic much branched possessing short soft erect hairs with distinctive smelling leaves. Leaves are undivided (simple), broad, egg/oval-shaped with a tapering tip (ovate) and very thick, they are pubescent (thickly studded with hairs), with the lower surface possessing the most numerous glandular hairs, giving a frosted appearances.^[16,18] It is used for the treatment of cough; maintain body heats Sore throats, Asthma Malarial fever, Mucus and Cancer Nasal congestion, Rheumatism, Flatulence, Skin ulceration, Diarrhea, Hepatoprotective.^[19,20]

MATERIALS AND METHODS

Collection of plant material

Plectranthus Amboinicus and Ocimum Tenuiflorum leaves were produced in spring season from Jangalakandriga locality in Muthukur in Nellore district. Leaves were identified and authenticated by botanist Dr. L. Rasingam at Botanical survey of India (BSI), Hyderabad.

Collection of test organism and preparation of stock culture

Middle brook 7H9 broth base M198

Middle brook 7H9 broth base with added enrichment is recommended for cultivation and sensitivity testing of mycobacterium tuberculosis. Firstly microorganism was provided by the Dr. U. Mohans lab Guwahati, Assam. Microorganism were investigated namely mycobacterium tuberculosis (M. segmatis). Then we prepared media for bacterial culture growth. Add 2.35gm of agar in 450 ml of distilled water and add 1ml of glycerol heat if necessary to dissolve the medium completely. Sterilize by autoclave for 10min. cool to 45⁰C or below and aseptically add contents of 1 vial of middle brook ADC growth supplement. Mix well before dispensing. Then inoculate the microbe in the media. Incubate it for 24-48 hrs in the incubator at 37⁰C.

Extraction Process

Extraction of Ocimum Tenuiflorum

Collect the leaves from the plant then dried and powdered it. 15gms of the dried leaf powder was loaded in the thimble of Soxhlet apparatus. It was fitted with appropriate size round bottom flask with 250ml absolute methanol and ethanol in separate flasks and upper part was fitted with condenser. After completion of extraction, the extract in round bottom flask was transferred into clean universal tubes. Universal tubes containing extracts were weighed and noted down and finally, the percentage yield was calculated.^[21]

Extraction of *Plectranthus Amboinicus*

Collect the leaves from the plant then dried and powdered it. 15gms of the dried leaf powder was loaded in the thimble of soxhlet apparatus. It was fitted with appropriate size round bottom flask with 250ml methanol and ethanol in separate flasks and upper part was fitted with condenser. After completion of extraction, the extract in round bottom flask was transferred into clean universal tubes. Universal tubes containing extracts were weighed and noted down and finally, the percentage yield was calculated.^[22,23]

Phytochemical Screening Tests

Chemical tests were carried out using an aqueous extract to identify various constituents using standard methods.^[24]

Test for Tannins

About 2ml of aqueous extract was stirred with 2ml of distilled water and few drops of FeCl_3 solution were added. Formation of green precipitate was indication of presence of tannins.

Test for Saponins

About 5ml of aqueous extract was shaken vigorously with 5ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Phlobatannins

About 2ml of aqueous extract was added to 2ml of 1% Hcl and the mixture was boiled. Deposition of red precipitate was taken as an evidence for the presence of phlobatannins.

Test for Flavonoids

About to 1ml of aqueous extract add 1ml of 10% lead acetate solution was added. The formation of yellow precipitate was taken as a positive test for flavonoids.

Test for Terpenoids

About 2ml of organic extract was dissolved in 2ml of chloroform and evaporated to dryness; 2ml of concentrated sulphuric acid was then added and heated for about 2min. Development of grayish colour indicates the presence of terpenoids.

Test for glycosides**Libermanns test**

About 2ml of the organic extract was dissolved in 2ml of chloroform and then 2ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully, a colour change from violet to blue green indicates the presence of a steroidal nucleus.

Test for Steroids

- 1) A red colour produced in the lower chloroform layer when 2ml of organic extract was dissolved in 2ml chloroform and 2ml concentrated sulphuric acid was added in it indicates the presence of steroids.
- 2) Development of greenish colour when 2ml of the organic extract was dissolved in 2ml of chloroform and treated with sulphuric acid and acetic acid indicates the presence of steroids.

Test for Alkaloids**Dragendorffs test**

2ml of extract and add few drops of Dragendorffs reagents to form green precipitate is observed.

Wagers test

2ml of extract and add few drops of Mayers reagent to form cream colour precipitate is observed.

Hagers test

2ml of extract and few drops of Hagers reagent to form yellow precipitate are observed

Antimicrobial Screening Method**Cup and plate method or Disc diffusion method**

The antimicrobial activity of each plant extracts was determined on Mycobacterium Tuberculosis [M.segmatidis] a disc diffusion method. The appropriate 7H9 middle brook growth supplement media was inoculated with inoculum [1×10^8 cfu/ ml] and poured into the petriplate up to 3mm diameter. After solidification of media make holes in those plates. Then add methanol and ethanol extracts in separate holes with two different concentrations as a test sample. Along with that add standard drug Isoniazid against tuberculosis in other hole as a

control. Then inoculate the inoculum in plate by spread plate method. The plates were incubated overnight at 37⁰c for 24-48 hrs in the incubator. Microbial growth was determined by measuring the diameter of zone of incubation with the help of inhibition zone scale.^[25,26]

RESULTS AND DISCUSSION

Table 1: Phytochemical screening of Plant extract with different solvent.

ESTS	Plectranthus amboinicus with Methanol	Plectranthus amboinicus with Ethanol	Ocimum Tenuiflorum with Methanol	Ocimum Tenuiflorum with Ethanol
Tannins	Failed	Passed	Passed	Failed
Saponins	Failed	Failed	Failed	Failed
Phlobatannins	Passed	Pass	Failed	Failed
Flavonoids	Passed	Passed	Passed	Passed
Terpenoids	Passed	Failed	Passed	Passed
Glycosides	Passed	Passed	Failed	Passed
Alkaloids	Passed	Passed	Passed	Passed
Steroids	Passed	Passed	Passed	Passed

Table 2: Growth of inhibition of Plectranthus Amboinicus and Ocimum Tenuiflorum.

S. No	Name of the Organism	Solvents	Plant Name	Zone of Inhibition [mm]
1.	Mycobacterium Segmatis	Ethanol	Plectranthus Amboinicus	7mm
		Methanol	Plectranthus Amboinicus	5mm
		Ethanol	Ocimum Tenuiflorum	7mm
		Methanol	Ocimum Tenuiflorum	5mm

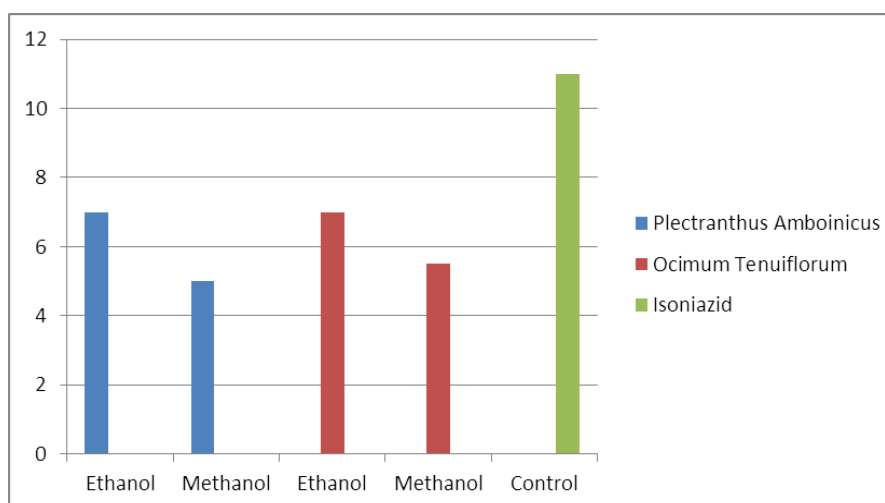


Fig. 1: Growth of inhibition of Plectranthus Amboinicus and Ocimum Tenuiflorum.



Fig. 2: Growth inhibition of methanolic extract of *Ocimum Sanctum* against mycobacterium tuberculae.



Fig. 3: Growth inhibition of ethanolic extract of *Ocimum Sanctum* against mycobacterium tuberculae.



Fig. 4: Growth inhibition of ethanolic extract of *Plectranthus Amboinicus* against mycobacterium tuberculae.



Fig. 5: Growth inhibition of methanolic extract of *Plectranthus Amboinicus* against mycobacterium tuberculae.

The qualitative phytochemical analysis was performed for the detection of Alkaloids, Steroids, Flavonoids, Tannins, Saponins, Terpenoids and Glycosides.^[27] In vitro antimicrobial activity was examined for aqueous and methanol and ethanol extracts of *Ocimum Sanctum* and *Plectranthus Amboinicus*. The antimicrobial assay was performed by agar disc diffusion method for solvent extract.

Qualitative phytochemical investigation discovered presence of steroidal, alkaloids and flavonoids for *Plectranthus Amboinicus* and *Ocimum Tenuiflorum* with ethanol and methanol leaf extracts phytochemical studies.

Phytochemical constituents such as compound are secondary metabolites of plants that serve a defense mechanism against predation by many microorganisms worked on steroidal

extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates metabolite alkaloids which are one of the largest groups of phytochemicals in the plants were observed in the all of extract of *Ocimum Sanctum* and *Plectranthus Amboinicus*.

Herbs are have tannins as their astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery.

Antibacterial activity

Whereas for *Plectranthus Amboinicus* with ethanol shows 7Mm zone of inhibition for *M.segmatis* for *Plectranthus Amboinicus* with methanol shows 5Mm zone of inhibition for *M.segmatis* for *Ocimum Tenuiflorum* with ethanol shows 7Mm zone of inhibition for *M.segmatis* for *Ocimum Tenuiflorum* with ethanol shows 7Mm zone of inhibition for *M.segmatis*. All these zone of inhibition of leaf extracts were compared with control Isoniazid shows 11mm for *M.segmatis*.

The results showed the *Plectranthus Amboinicus* and *Ocimum Tenuiflorum* leaves ethanol extract shows good inhibition against *M.segmatis*. The observed antimicrobial activity could be explained by the fact that plant extract may attach to the surface of the cell membrane disturbing permeability and expression of function of the cell. The interaction of the plant extract with microbial cytoplasmic components and nucleic acid inhibits the respiratory chain enzymes and interferes with the membrane permeability. It is also possible that extract not only interact with the surface of the membrane but also penetrates inside the bacteria.

CONCLUSION

Here we concluded that the *Plectranthus Amboinicus* and *Ocimum Tenuiflorum* leaves extract contains alkaloids, steroids, glycosides, flavonoids and tannins and possess antimicrobial potential against acid fast bacteria, *M.segmatis*, it is therefore confirmed as a useful antimicrobial agent. The present study provides evidence that ethanol extract of *Ocimum Tenuiflorum* and *Plectranthus Amboinicus* contains medicinally important bioactive compounds than Methanol extract and this justifies the use of plant species as traditional medicine for treatment of tuberculosis and other diseases.

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