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# TESTING OF ANTI-MYCOBACTERIAL ACTIVITY & PHYTOCHEMICAL PROFILING OF *Glycyrrhiza uralensis, Pipper longum, Allium sativum, Zingiber officinale, Curcuma longa* against *Mycobacterium smegmatis*

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## ABSTRACT

The aim of this study is to add knowledge and to identify the plants that could be sources of lead compounds for new tuberculosis drug development and to check the potentiality of anti-mycobacterium activity, investigation of bioactive compounds contained in medicinal plant showing the anti-tubercular activity in selected medicinal plants. The aqueous and alcoholic extracts of *Glycyrrhiza uralensis, Pipper longum, Allium sativum, Zingiber officinale, Curcuma longa* were studied. The result encouraged as the *Allium sativum* have the potentiality of anti-mycobacterium (anti-tubercular) activity.

***Key words:*** *Anti-mycobacterium activity, tuberculosis, anti-tubercular, bioactive*

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**INTRODCTION:**

In the recent years, research on medicinal plants has attracted a lot of attentions globally. Evidences has been accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, having antimicrobial properties. *(Dahanukar S. A et. al, 2000 & Cowan M M, 1999)* Clinical microbiologists have two reasons to be interested in antimicrobial plant extracts. First, these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested in humans. Scientists realize that the effective life span of any antibiotic is limited, so new sources especially plant sources are also being investigated. Second the public is becoming aware of the problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. (*Cowan M M, 1999*)

Tuberculosis is a highly infectious disease, infected 33 percent of the world's population including 40 percent from India. The problem becomes very much serious as *Mycobacterium tuberculosis* developed resistance against both the first line drugs (Isoniazid, Rifampicin, Pyrazinamide, Streptomycin and Ethambutonal); which are usually used for the treatment of TB patients with susceptible *Mycobacterium tuberculosis* as well as the Second-line drugs (Kanamycin, Ethinoamide and Cycloserine etc.); have many more adverse effects that the first-line anti-TB drugs. Due to this, there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* all over the world including India and there is an urgent need to search for newer anti-tuberculosis compounds/drugs. In 1993, the World Health Organization (WHO) declared TB to be a global emergency. (*Arya Vikrant, 2011*) The exact cause of this is unknown, although it is thought that it could be because of the resurgence of TB due to HIV infection as well as MDR-TB due to inefficient management. (*Heinrich M. et. al., 2001*). Medicinal plants offer a great hope to

fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. *(Heinrich M. et. al., 2001).*

India is one of the few countries in the world which has unique wealth of medicinal plants. So far, few plants have been tested against mycobacteria and a few plants which showed anti-TB activity were *Salvia hypargeia, Euclea natalensis*, *Allium cepa, Alliun sativum*

*etc*. Not only in India, anti-tubercular plants were found all over the world including South Africa, New Zealand, Malaysia, Nigeria, Tibet *etc. (Arya Vikrant, 2011)*

Natural products as crude materials with efficacy against various diseases have been selected by humans over many generations of practical experience. *(Navarro et. al, 2003; Mbata & Saikia, 2005; Emma et. al., 2010; Renu et. al., 2010; Narwadia et. al, 2011)*

**MATERIALS AND METHODS:**

**Collection of plants:** The medicinal plants used for study viz. the dried fruits of Long pepper- *Pipper longum,* dried stem of Mulethi- *Glycyrrhiza uralensis,* bulbs of Garlic- *Allium sativum,* roots of Ginger- *Zingiber officinale &* Haldi- *Curcuma longa* were purchased from the local market of Agra.

**Drying and pulverizing of fresh medicinal plants:** The bulbs of Garlic- *Allium sativum,* roots of Ginger- *Zingiber officinale* were collected fresh, macerated and dried under shade inside the room to avoid direct sunlight that could degrade some of the compounds in the plants. They were also spread out and regularly turned at regular interval to avoid fermentation and rotting. The dried parts were pulverized using pestle and mortar/grinder. The powder was weighed and stored in air tight jars at room temperature.

**Pulverizing of dried medicinal plants:** The fruits of *Pipper longum,* stem of *Glycyrrhiza uralensis, & Curcuma longa* were pulverized by using pestle and mortar. The powder was weighed and stored in air tight jars at room temp.

**Plant extracts preparation:** For each medicinal plant sample, approximately 1gm. of dried plant tissue was placed in 50 ml conical flasks. Sterile distilled

water was added to the sample to give the final concentration of 100 mg/ml. The samples were incubated at $55^0$C for 1 hour and after cooling at room temperature they were stored at $-40^0$C. The incubation at $55^0$C is because some traditional medicines were prepared at this temperature. The resulting crude extracts were sterilized using a 0.22 µm filter paper prior to anti-microbial analysis. *(Bansod S. & Rai M., 2008)*

**Media for *Mycobacterium smegmatis*:** Medium is prepared by utilizing L.R. / A.R. grade chemicals and all the media were sterilized by autoclaving at 15 psi for 20 minutes. The Luria Broth agar medium supplemented with Tween-80, Kanamycin & D-arabinose was used for the maintenance of standard culture of *Mycobacterium smegmatis.*

**Anti-mycobacterium Assay by Disc Diffusion Technique:** The anti-mycobacterial activity is determined by Disc Diffusion Method in which the sterile discs (5mm diameter, Whatmans filter paper No.42) were soaked in crude extracts of selected medicinal plants. The test was performed in triplicate. These dishes were incubated for 48 hour at 28ºC onto the Petri plates containing Luria Broth agar medium supplemented with Tween-80, Kanamycin & D-arabinose inoculated with *Mycobacterium smegmatis* and zone of inhibition in mm were determined after 48 hour. *(Bansod & Rai, 2008)*

**Identification tests for active compounds:** The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure.

**Test for alkaloids:** The presence of alkaloids was determined by dissolving and filtering 200 mg plant extract in 10 ml methanol followed by filtration using Whatmann filter paper no. 42 (125 mm) filters. One thousand microlitres (1 ml) of the filtrate was then mixed with 6 drops of Wagner's reagent. Creamish, brownish-red or orange precipitate indicated the presence of alkaloids. A low (+) reaction was recorded if the addition of the reagent produced a faint turbidity; a moderate (++) reaction was recorded if a light opalescence precipitate was observed; and a high (+++) reaction was recorded if a heavy yellowish-white precipitate was found. ***(Mariita et. al., 2010)***

**Test for cardiac glycosides:** Five millilitres (5 ml) of each extract were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides. A (+) reaction was recorded when a faint green-blue colour was observed (indicating low concentrations of detectable cardiac glycosides); a (++) reaction was recorded when a medium green-blue colour was observed (indicating moderate concentrations of detectable cardiac glycosides); and a (+++) reaction was recorded when a deep green-blue colour was observed (indicating high concentrations of detectable cardiac glycosides). ***(Mariita et. al., 2010)***

**Test for flavonoids:** Five millitres of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2S0_4$. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. A (+) reaction was reported for pale yellow colour; (++) for moderate yellow and (+++) for strong yellow colouration, indicating low, moderate and high concentrations of flavonoids, respectively, in the plant extract. ***(Mariita et. al., 2010)***

**Test for saponins:** To 0.5 g of the extract was added 5 ml of distilled water in a test tube. The solution was shaken vigourously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion. A (+) sign was recorded when the froth reached a height of 0.5 cm; a (++) sign for a height of 0.6 - 1 cm; and a (+++) sign for a height of more than 1cm, indicating low, moderate or high concentrations of saponins, respectively, in the plant extract. ***(Mariita et. al., 2010)***

**Test for tannins:** About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride were added and observed for brownish green or a blue-black colouration. A (+) reaction was recorded when a slight precipitate was observed; a (++) reaction was recorded when a medium precipitate was observed; and a (+++) reaction was

recorded when a heavy precipitate was observed. The reactions were used to indicate the presence of different concentrations of detectable tannins, with (+) representing low, (++) moderate and (+++) high levels of tannins. ***(Mariita et. al., 2010)***

**RESULTS AND DISCUSSION:**

**Anti-mycobacterium assay using aqueous plant extracts in supplemented LB agar medium using *Mycobacterium smegmatis:*** Zone of inhibition at 1% w/v, 2% w/v, 3% w/v, 4% w/v, 5% w/v and 6% w/v of aqueous extracts of medicinal plants had been analyzed in triplicates. Out of 5 medicinal plants *Allium sativum* shows the maximum zone of inhibition and $MIC_{50}$ of 27.67 mm at 4% w/v and <2% w/v respectively followed by *Pipper longum* with zone of inhibition and $MIC_{50}$ of 16.33 mm at 4% w/v and <2% w/v respectively, *Curcuma longa* shows the maximum zone of inhibition and $MIC_{50}$ of 15.5 mm at 5% w/v and >2% w/v respectively while *Glycyzrrhiza glabra* and *Zingiber officinale* did not show any significant inhibition against *Mycobacterium smegmatis*. The results are shown in Table 1.

| **Botanical name of Medicinal Plants** | **(Mean of Zone of inhibition in mm ± S. D.) at different concentrations** | | | | | |
|---|---|---|---|---|---|---|
| | **1%** | **2%** | **3%** | **4%** | **5%** | **6%** |
| *Glycyzrrhiza glabra* | ND | ND | ND | 4.33 ± 0.58 | 6.33 ± 0.58 | 5.83 ± 0.29 |
| *Allium sativum* | 12.67±0.58 | 17.00 ± 1.00 | 24.00 ± 2.00 | 27.67 ± 0.58 | 27.67 ± 0.58 | 27.67 ± 0.58 |
| *Pipper longum* | 6.33 ± 0.58 | 8.33 ± 0.58 | 11.33 ± 0.58 | 11.33 ± 0.58 | 16.33 ± 0.58 | 16.00 ± 1.0 |
| *Zingiber officinale* | 5.00 ± 0.00 | 7.50 ± 0.50 | 9.83 ± 0.29 | 9.83 ± 0.29 | 11.66 ± 0.58 | 11.66 ± 0.29 |
| *Curcuma longa* | 6.33 ± 0.58 | 7.17 ± 0.29 | 10.83 ± 0.76 | 10.83 ± 0.76 | 15.50 ± 0.50 | 15.33 ± 0.58 |

**Table 1:** Anti-mycobacterium assay using aqueous plant extracts in supplemented LB agar medium using *Mycobacterium smegmatis*

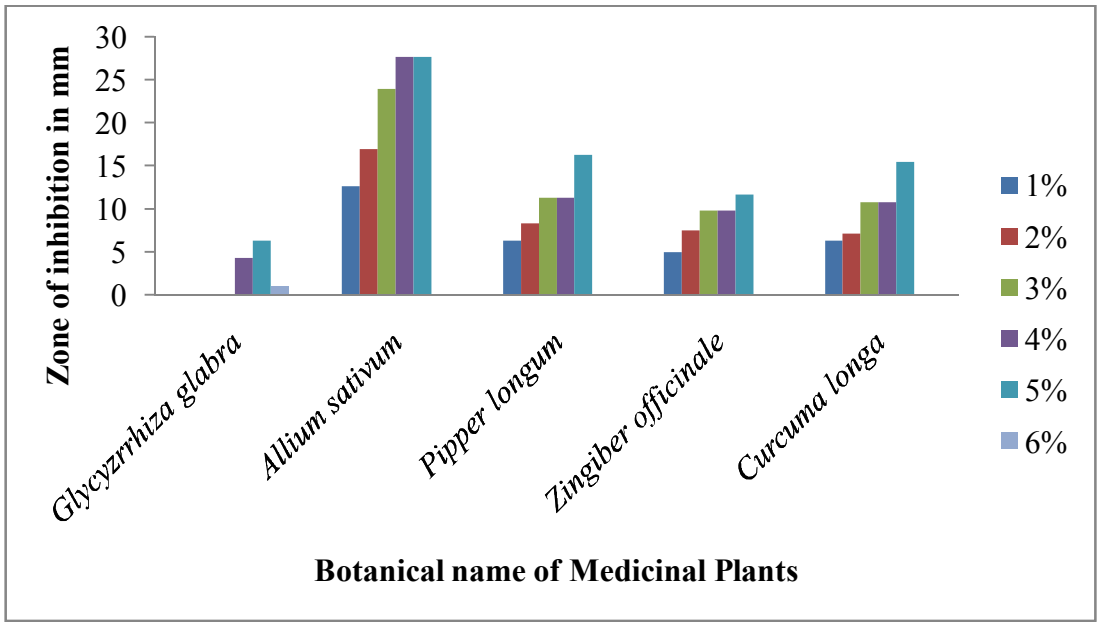


**Figure 1:** Anti-mycobacterium assay using aqueous plant extracts in supplemented LB agar medium using *Mycobacterium smegmatis*

| **Medicinal Plants** | ***Glycyzrrhiza glabra*** | ***Allium sativum*** | ***Pipper longum*** | ***Zingiber officinale*** | ***Curcuma longa*** |
|---|---|---|---|---|---|
| **$MIC_{50}$** | - | <2 | <2 | <2 | >2 |
| **Lethal value** | - | 4 | 5 | 5 | 5 |

**Table 4:** $MIC_{50}$ and lethal value of medicinal plants in aqueous & alcoholic extracts

**Identification tests for active compounds**:

All the studied aqueous plant extracts were analyzed for the presence of alkaloids, glycosides, Flavonoids, Saponins & Tannins as per method described in material and method. The results are summarized in table 5.

| Name of plant | Alkaloids | Glycosides | Flavonoids | Saponins | Tannins |
|---|---|---|---|---|---|
| *Glycyzrrhiza glabra* | + | – | – | +++ | – |
| *Allium sativum* | + | ++ | – | +++ | ++ |
| *Pipper longum* | + | – | + | +++ | +++ |
| *Zingiber officinale* | + | – | – | +++ | ++ |
| *Curcuma longa* | + | + | – | +++ | ++ |

**Table 5: Observation table for identification tests for active compounds**

**Conclusion:** These results give an indication that *Allium sativum* & *Pipper longum* have the potentiality of anti-mycobacterium (anti-tubercular) activity and may be used for further study.

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