



## ***IN – VITRO ANTI TUBERCULAR ACTIVITY OF FLOWERS OF COURROUPITA GUANENSIS L.***

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### **ABSTRACT**

Modern civilization is facing hundreds of disorders associated with microorganisms. The natural phytochemicals from non-edible plants are gaining importance to fight against these disease. The intention of this study is to evaluate the ethanol and dichloromethane extracts of flower of *Couroupita guianensis* (Lecithydaceae) for anti-tubercular activity. The anti-tubercular activity of all the extracts of *Couroupita guianensis* have been evaluated against *Mycobacterium tuberculosis* H73Rv strain using Microplate Alamar Blue Assay (MABA). The activity was documented within MIC range of 0.8 to 100µg/ml. The results of MABA showed that both ethanol and dichloromethane extract exhibited significant anti-tubercular activity. The present investigation suggests that *Couroupita guianensis* possess remarkable anti-tubercular activity.

### **INTRODUCTION**

There are many medicinal plants all over the world. The drug which is obtained from these plants has lesser side effects. Medicinal plants are age long agents for human beings as they were having many therapeutic values. Now a days plants are playing a crucial role in drug extraction. Plants are economically essential [1]. They contain many constituents that are useful in treatment of many human diseases. Plants are having rich secondary metabolites that are having most therapeutic use for many ailments. Some plants extracts are also used to prevent the antibiotic resistance. *Couroupita guianensis* is a large deciduous evergreen plant having a great medicinal value. It is having a height of 20 meters. Leaves are alternate oblongate up to 20 centimeters long. It shows racemose inflorescence arising from the trunk and large branches. Flowers are reddish with a yellow tinge on the outside, fragrant with stamens borne on an overarching androphore. Fruit is a large, reddish brown globose, 15-24 cm with woody capsule containing 200-300 seeds each. Each and every part of the plant is having the medicinal value. Tuberculosis (TB) is one of the leading infectious disease and

health burden in the world. It has been estimated that, one third of world's population including 40% from India estimated to be infected with tuberculosis. The leaves of *Couroupita guianensis* used as an anti-depressant using in rats. The flowers extract of *Couroupita guianensis* has different pharmacological properties. Among them the most important activity is Anti tubercular activity as the disease Tuberculosis is the most threatening disease in the present scenario in the world [2].

*Couroupita guianensis* has showed a broad spectrum of anti-bacterial and antifungal activities. The leaves of *Couroupita guianensis* yielded an aliphatic triterpene, it is used as an anti-depressant using in rats. The phenolic compounds of *Couroupita guianensis* are active in curing the kidney and stomach problems and helpful as anti-inflammatory in action [3]. The biological function of flavonoids, apart from their anti-oxidant properties, includes protection against allergies, inflammation, platelet aggregation, microbial ulcers, hepatotoxins virus and tumours. It is known that one of the active constituents of the medicinal plant *Couroupita*

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*guianensis* namely isatin, is known to exert cytotoxic activity against certain cancer cell lines, being a potential source of new chemo therapeutic agents. In a study performed using extracts of *Couroupita guianensis* against human promyelocytic leukemia (HL60) cells, isatin showed antioxidant activity and was cytotoxic to the HL60 cells due to induction of apoptosis, a natural cell death. These results suggest that isatin can be further evaluated to be used as a prophylactic agent to prevent the free radical induced cancer and as a chemotherapeutic agent to kill the cancer cells [4].

The intention of this study is to evaluate the ethanol and dichloromethane extracts of flower of *Couroupita guianensis* (Lecithydaceae) for antitubercular activity.

## EXPERIMENTAL METHODS

### Collection and processing of plant material

The flowers are collected from the plant *Couroupita guianensis* and they are dried under the shade for 2 weeks and they are powdered finely into powder. The powder was passed through sieve number 40 and the obtained powder was collected.

### Extraction of plant materials

The powder so obtained weighed 200g and divided into two equal parts and taken in two iodine flask they were added with ethanol and dichloromethane respectively. The two flasks were kept on orbital shaker for 48 hours to obtain the extract. The solvents were evaporated and the two solvents extracts were obtained with the yield of 1.4% and 1.8% respectively.

### Microplate Alamar Blue assay (MABA)

The anti-mycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate

and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. [5-7]

## RESULTS AND DISCUSSION

The results obtained from the microplated almar blue assay for the assesment of anti tubercular activity was shown in figure no. 1. The minimum inhibitory concentration (MIC) of both ethanol and dichloromethane extracts exhibiting minimum inhibitory concentration 1.6µg/ml of respective extracts. The activity may be due to the presence of alkaloids and terpenoids present in the flowers of *Couroupita guianensis* [8-10].

Table 1: Inhibitory response of both the extracts of *Couroupita guianensis*

| Concentration | Ethanol extract<br>Response | Dichloromethane<br>extract Response |
|---------------|-----------------------------|-------------------------------------|
| 0.8           | N                           | Y                                   |
| 1.6           | N                           | Y                                   |
| 3.2           | Y                           | Y                                   |
| 6.4           | Y                           | Y                                   |
| 12.5          | Y                           | Y                                   |
| 25            | Y                           | Y                                   |
| 50            | Y                           | Y                                   |



Figure 1: Microplate shows minimum inhibitory concentration of ethanol and dichloro methane extracts of *Couroupita guianensis*

## CONCLUSION

The activity was documented within MIC range of 0.8 to 100µg/ml. The results of MABA showed that both ethanol and dichloromethane extract exhibited significant antitubercular activity at 0.8 µg/ml of ethanol extract showed the inhibition and 3.126µg/ml of dichloromethane extract showed its inhibition. The present investigation suggests that *Couroupita guianensis* possess remarkable anti tubercular activity with increase in concentration of extract. This drug could be

considered for isolation of responsible bioactive molecule for anti tubercular activity.

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