



**EVALUATION OF ANTIFUNGAL, ANTITUBERCULOSIS AND ANTIOXIDANT  
ACTIVITIES OF *BLUMEA LACERA* AND *CYATHOCLINE PURPUREA* (ASTERACEAE)**

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**ABSTRACT**

Two weed species i.e. *Blumea lacera* and *Cyathocline purpurea* were selected for evaluation of their antimicrobial activities. Plant extracts in their different concentrations were examined for antifungal activity, antioxidant activity and antituberculosis activity. Antifungal activity of both the species were studied with the help of MIC method against *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum*. Antioxidant activity was performed by DPPH assay method using standard ascorbic acid and DPPH solution. While antimycobacterial activity of extracts were assessed against *M.tuberculosis* using MABA i.e. Microplate Alamar Blue Assay method. From all the three activities it was observed that weed extracts in different solvents are more sensitive for performed biological activities. At minimum concentration within the range of 200-300 ppm of concentration of extracts it inhibits fungal growth. Effect of radical scavenging activity in percentage also studied by using DPPH method. From antituberculosis activity different concentration of weed extract in µg/ml were studied for their sensitive and resistant nature. From these three activities it proves that both weed species shows better biological activities.

**KEYWORDS:** *Blumea lacera*, *Cyathocline purpurea*, antifungal, antioxidant, antituberculosis.

**INTRODUCTION**

Plants which are used for estimation of heavy metals are from the Asteraceae family, they shows close resemblance with medicinal properties and commonly found in local region as a weed. *Blumea lacera* is an erect villous herb and their lower leaves are petioled, often incised or lyrate and the upper sessile, elliptic oblong or obovate, having finely silky pubescut. This plant is astringent, antispasmodic, stomachic, antipyretic as well as diuretic, cures bronchitis, fever, and also burning sensation. Leaf juice is anthelmintic and stimulant, mixed with pepper and it is given in treatment to cure piles. Roots mixed with pepper are given for the treatment of cholera and along with rhizomes of *Cyprus rotundus* also given in dysentery. An alcoholic extract of the herb exhibited marked anti-inflammatory activity. *Blumea lacera* is described as a valuable medicinal plant in many vital systems of medicines including ayurveda homeopathy and Yunani or unani.<sup>[1]</sup> *Cyathocline purpurea* is an annual and occasionally perennial. It is found growing in water logged soils. The plant used in medicines, the roots are used in treating stomach pain. This Indian medicinal plant is an erect, delicate, gregarious, glandular, hairy, annual or biennial herb. It grows upto 20 to 25cm in height. The stems of this plant are reddish or purple tinged, and branched from the base. The leaves are sessile, upper leaves are small in size,

segments are serrate and are covered with thin hair. The flowers are usually purple in colour and occur in corymbs at the end of branches.<sup>[2]</sup> *Blumea lacera* and *Cyathocline purpurea* are the plants which shows various medicinal uses against some diseases and uncomfot conditions. Some heavy metals are analyzed from these two plant samples for the purpose of studying the presence of their quantitative factor. Heavy metals are present in varying concentration in different plants.<sup>[3]</sup>

*Blumea lacera* and *Cyathocline purpurea* weed plants were collected and their extracts were prepared in organic solvents like methanol, petroleum ether, hexane and acetone. These extracts contains desirable active compounds and were analyzed with the help of biological activities. The activities for which extracts were evaluated are antifungal, antituberculosis and antioxidant activity. For antifungal activity the alcoholic solution of the plant extract can be examined on fungal infection, which shows better antifungal activity. To study this activity extracts which are constituted different solvents was assessed using Mycelium Inhibition Concentration Method i.e. MIC against three different phytopathogenic fungi, viz. *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum*. The MIC values indicate that this plant extracts has great effect on selected fungal pathogens.

*Blumea lacera* has significant medicinal uses. Methanolic extract of *Blumea lacera* shows better result for biological activities like cytotoxic nature capacity, antifungal activity, anti bacterial activity, anti pyretic activity, anti viral and anti diarrhoeal activity.<sup>[4]</sup> Traditional use of *Blumea lacera* is as a antifungal agent, antiviral active component, antihelminthic as well as to cure dysentery also.<sup>[5]</sup> *Cyathocline purpurea* extract also shows biological activities like analgesic activity (peripheral analgesic effect), anti-inflammatory activity, and antioxidant activity by using H<sub>2</sub>O<sub>2</sub> radical scavenging assay method. These activities have been observed on rodents. Hence plant extract has many medicinal uses on living beings.<sup>[6]</sup> In pharmacognostic extraction of *Cyathocline purpurea* it has a strong odour, it contains aromatic compounds and has fibrous texture, it is bitter to taste. *Cyathocline purpurea* in their herbal formulation are used for medicinal treatments in human beings.<sup>[7]</sup>

So it proves that both weed species shows biological activities at greater extent. In the screening of both species phytochemical constituents and heavy metals as well as essential nutrients are also evaluated which may be able to their effects on living beings as a biologically active agent also for medicinal use. This evaluation mostly focus on plant extracts in different solvents. Their biological activity at different concentrations has been observed which will provide further detail information about biological active nature of both species. Hence along with all performed activities which was done earlier, this work focuses on related activities with standard methods.

## MATERIAL AND METHODS

The biological activities in plant tissues can be evaluated in two plant species such as *Blumea lacera* and *Cyathocline purpurea*, for their analysis, plant extract obtained from whole dried sample was used. Total plant analysis for biological activities are more useful in nature and is more reliable. Plant sample collected from local area (Kolhapur region). After collecting both fresh plant samples, they get separated from dirt and adhered soil as well as followed by washing and cleaning under water flow to remove stucked dust. The whole fresh clean plant samples were dried by introducing to direct sunlight. Again these air dried samples were oven dried to remove total moisture content at temperature 60-70°C by maintaining constant weight. The dried plant materials were ground and sieved to get fine powder. These crushed sample powder get stored in two air tight containers and were used for further analysis. *Blumea lacera* and *Cyathocline purpurea* plant powder were used for extract preparation. In different solvents such as methanol, pet ether, hexane and acetone these extracts are prepared by using standard method. From separation techniques soxhlet extraction was used for extraction and is able to get more qualitative separation in terms of active constituents. Prepared extracts were used to study

their biological activities like antifungal activity, antituberculosis activity and antioxidant activity.

### A. Antifungal activity

Antifungal assay was performed by mycelium inhibition method. *Aspergillus Flavus*, *Alternaria alternata* and *Fusarium Oxysporium* these fungal species were grown against plant extract at concentration 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, 300ppm. About 20 ml of the medium (PDA) were poured into glass Petridishes. Each Petri-dish was inoculated at the center with a mycelial disc (6 mm diameter) taken at the periphery of *Alternaria alternata* and *Aspergillus flavus* colonies grown on PDA for 48 h while *Fusarium oxysporium* takes 36hrs to grow. Control plates were inoculated following the same procedure. Plates were incubated at 25°C for 8 days and the colony diameter was recorded each day. Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration in which no growth occurred. The inhibited fungal discs treated sets were re-inoculated into the fresh medium, and revival of their growth was observed. Diameter of fungal colonies of treatment and control sets was measured, and percentage inhibition of fungal growth was calculated according to following formula,

$$PI = 1 - \frac{Dt}{Dc} \times 100$$

Dt: The diameter of growth zone in the test plate

Dc: The diameter of growth zone in the control plate

### B. Antituberculosis activity

By using Microplate Alamar Blue Assay (MABA) method the anti mycobacterial activity of compounds from different extracts were studied against *M. tuberculosis*. This method is used because it is non-toxic technique also uses a thermally stable reagent and advantage of this technique is it is able to show good correlation with propotional and BACTEC radiometric method. 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 100µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final 100 to 0.2 µg/ml drug concentrations were tested. Covered plates were sealed with parafilm. This plates are incubated for 5days at 37°C. After that 1:1 mixture of Alamar Blue reagent and 10% tween 80 (25µl of freshly prepared) was added to the plates and kept for incubation (about 24 hrs). A blue and pink colors in the well was observed from which blue colour is able to show inhibited bacterial growth while pink color was shows growth of bacteria. In this method the MIC value shows exact minimum drug concentration which prevents the color change from blue to pink.<sup>[8]</sup>

**C. Antioxidant activity**

Antioxidant activity was performed by using DPPH assay. For this analysis 24mg DPPH in 100ml methanol were used as a stock solution. Standard ascorbic acid solution was prepared were taken in concentration 0 µl (i.e. control), 50, 100, 150, 200, 250, 300 µl and whole samples were make for the volume 1ml by addition of distilled water. Then in each sample 2ml of DPPH stock

solution was added and this sample allowed to set for 10min in dark. After this absorbance of samples were measured at 517nm. From absorbance values %RSC was calculated by using formula given below,

$$\%RSC = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

**RESULT AND DISCUSSION**

**A. Antifungal activity results**

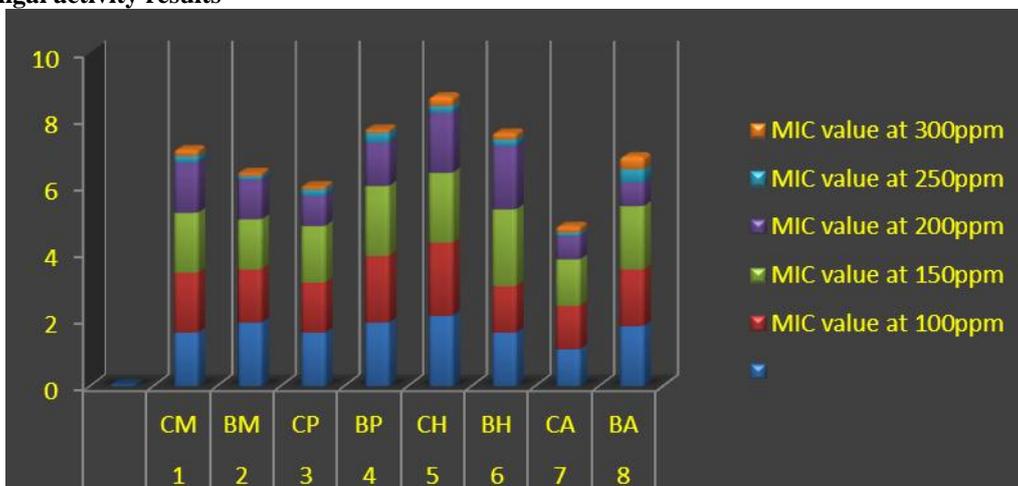


Fig. 1: MIC value for fungal species *Alternaria alternata* (C- *Cyathocline purpurea*, B- *Blumea lacera*, M- methanol, P- petroleum ether, H- hexane, A- acetone).

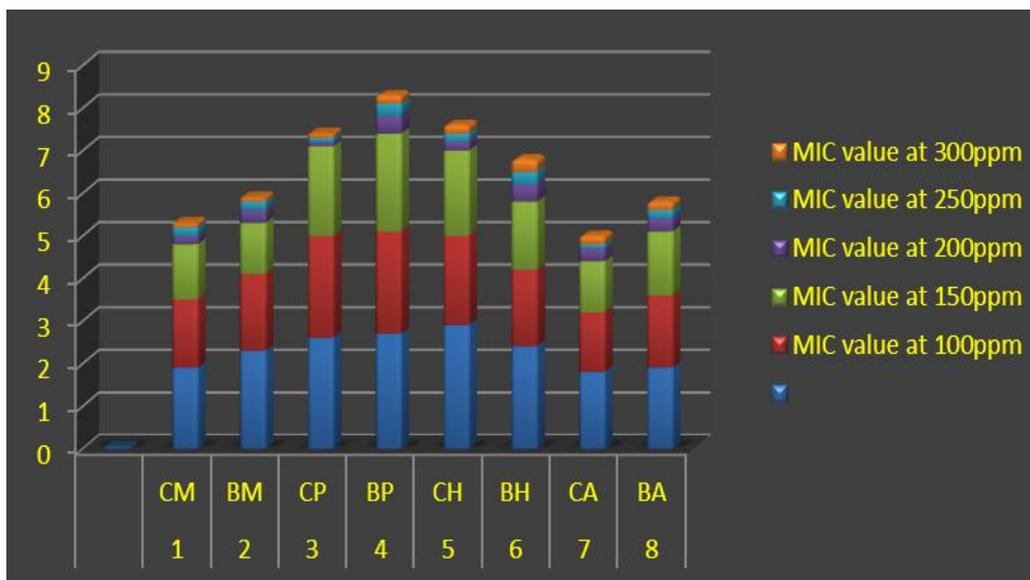


Fig. 2: MIC value for fungal species *Aspergillus flavus*.

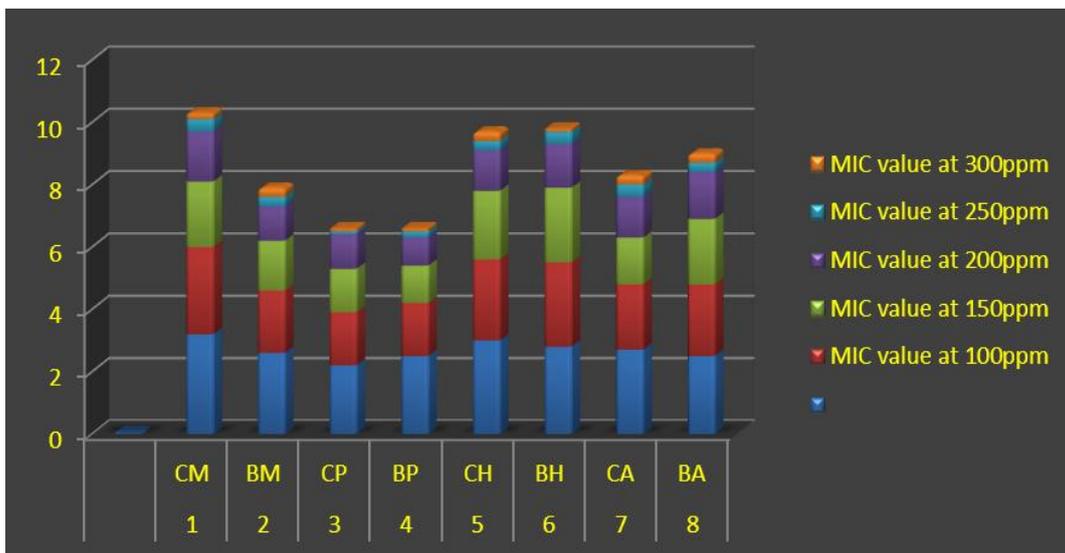


Fig. 3: MIC value for fungal species *Fusarium oxysporum*.

**B. Anti-tuberculosis activity results**

**Table 1: Antituberculosis activity sensitivity results.**

Sr. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	CM	S	S	S	S	S	S	S	R
2	BM	S	S	S	S	S	S	S	R
3	CP	S	S	S	S	S	S	S	R
4	BP	S	S	S	S	S	S	S	R
5	CH	S	S	S	S	S	S	R	R
6	BH	S	S	S	S	S	S	R	R
7	CA	S	S	S	S	S	R	R	R
8	BA	S	S	S	S	S	S	S	R
9	CW	S	S	R	R	R	R	R	R
10	BW	R	R	R	R	R	R	R	R

(Note: S–Sensitive, R–Resistant, Strain used: *M.tuberculosis* (H37 RV strain): ATCC No- 27294, Here are the standard values for the Anti-TB test which was performed, Pyrazinamide- 3.125µg/ml, Streptomycin- 6.25µg/ml, Ciprofloxacin- 3.125µg/ml).

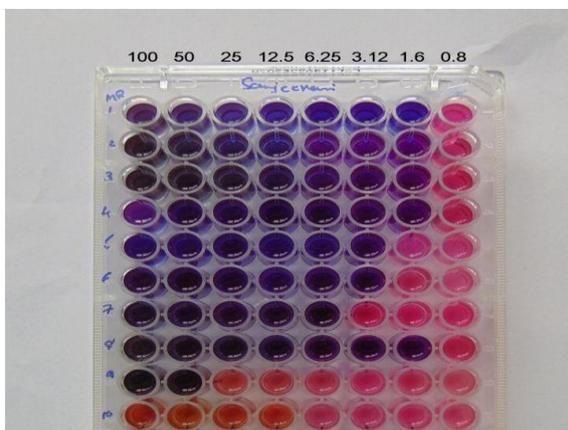


Fig. 4 a) Anti TB results photograph.

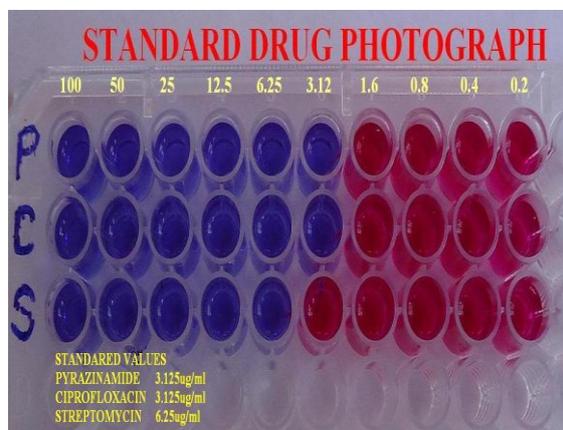


Fig. 4 b) Anti TB results (standard photograph).

### C. Antioxidant activity results

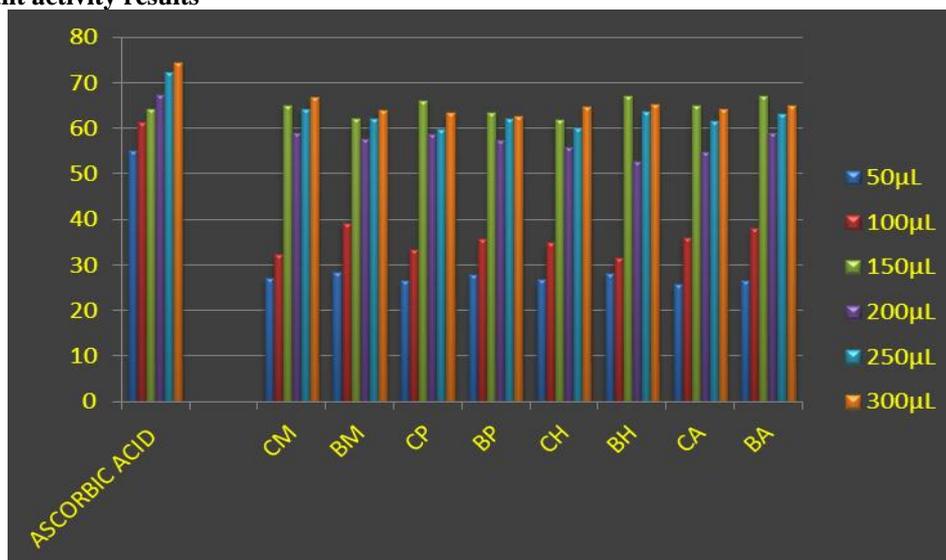


Fig. 5: Antioxidant activity by DPPH assay.

*Blumea lacera* were identified as medicinally important against headache, it is an aromatic herbal species, and also it shows anticancer activity.<sup>[9]</sup> *Blumea lacera* in solvents like methanol and petroleum ether shows presence of phytochemicals, in earlier investigation it was found that it has antidysentric as well as antidiarrhoeal properties because it contains phytochemicals. This species shows good cytotoxic nature and Antimicrobial activity.<sup>[10]</sup> *Blumea lacera* shows antioxidant activity at lowest concentration in methanol extract while pet ether extract shows antioxidant function at highest concentration. More reducing power is of methanol extract as compared to ascorbic acid.<sup>[11]</sup> It shows essential bioactive constituents and antimicrobial activity hence it is a valuable plant with reference to their medicinal values.<sup>[12]</sup> Through experimental study *Cyathocline purpurea* shows anti-inflammatory and analgesic properties. Chemical constituents exerts antioxidant anthelmintic, antimicrobial as well as anticancer properties. It gives relief on stomachache.<sup>[6]</sup>

*Blumea lacera* and *Cyathocline purpurea* both are weed species from asteraceae family and This evaluating study gives information that both the species shows good biological activities. Their extracts are more sensitive to antifungal, antioxidant and antituberculosis activities. Previously some activities were also studied but along with that biological activities which are recorded gives an idea about their medicinal values due to presence of bioactive constituents and phytochemicals. *Alternaria alternata* and *Fusarium oxysporum* fungal species shows inhibitory concentration at 250ppm of both plant sample extracts (Fig.1,3), while 200ppm concentration of sample extract inhibits the growth of *Aspergillus flavus* (Fig.2). In Anti-TB activity extracts of sample species in different solvents are compared with standard result (Fig.4 b). Anti tuberculosis activity for Methanol and pet ether extract of *Blumea lacera* and *Cyathocline purpurea*

shows sensitive nature at low concentration 1.6 µg/ml (Fig.4) hexane and acetone extract gives more sensitive nature at concentration in between 3.12 to 6.25 µg/ml (Fig.4), but water extract of samples shows more resistant nature at higher concentration also. So this investigation informs that water extract is unable to show medicinal properties at low concentration (Table 1). DPPH assay shows good result for antioxidant activity at minimum concentration 150 µl as it compared with standard ascorbic acid (Fig.5).

### CONCLUSION

From this observations, results identified the biological active nature of *Blumea lacera* and *Cyathocline purpurea* hence it proves that both species contains significant biological constituents which focuses on their medicinally important value. Along with all activities which were studied earlier by other researchers it shows also antituberculosis activity which was not done before. This investigation focuses on biological activities but it also found that presence of phytochemicals shows active and sensitive nature of species and gives good result for antifungal, antioxidant and antituberculosis activity.

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