

Research Article



Evaluation of Antibacterial Activity of Extracted Oil from *Blumea lacera* and *Cyathocline purpurea* (Asteraceae)

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ABSTRACT

The Asteraceae family of plants is a broadly spread medicinal plant throughout the world and has been used while early time. Members of this family have been used conventionally as for medicinal uses. Phytochemical analysis of the Asteraceae family have exposed that many mechanism from this family are highly bioactive in nature. From Asteraceae family many plants are the source of different kinds of medicinal raw material and it includes active constituents which make substances for drugs. In the Russia to obtain the mandatory number of high quality medicinal raw material these plants are cultivated ever more. From these family *Blumea lacera* and *Cyathocline purpurea* these weeds were selected to study their biological active nature to observe Antibacterial activity. Essential oil was extracted from *Blumea lacera* and *Cyathocline purpurea* by using Clevenger apparatus. Fresh extracted oil was studied for their antibacterial activity. One bacterial species from agricultural field i.e. *Pseudomonas aeruginosa* (NCIM 5032) and one bacteria as a human pathogen i.e. *Bacillus subtilis* (NCIM 2635) were studied for this activity. Inhibition zone was measured in mm.

Keywords: *Blumea lacera*, *Cyathocline purpurea*, antibacterial activity, *Pseudomonas aeruginosa*, *Bacillus subtilis*.

INTRODUCTION

Indian Weeds Used As Folk Medicine

India is rich in Biological diversity and is gifted with many useful and functional plants. About 250 weed species are well identified and known in the total 2,50,000 plant species. These weeds have non nutritive plant chemical that include defensive disease preventing compounds against various bacteria and microorganisms.¹ Weeds are commonly used for easy processes in environmental balance such as it helps to prevent soil erosion, it maintains nature balance related to environmental factors, it acts as a manure and it is able to cause biodegradation it may also use as a organic matter or organic manure *Xanthium strumarium*. Some weeds are also used as a leafy vegetables *Amaranthus viridis*, and also as a fodder crops for some livestock species. Also weed species shows medicinal importance *Leucas aspera*. Some species are economically important like *Saccharum spontaneum*. *Lantana camara* like species exhibit beautiful phenomenon for their flowers. Hence term 'Weed' is not limited to harmful causes but it has so many naturally exhibiting valuable properties.

The creation of bioactive secondary metabolites or some phytochemicals by plants is frequently an evolutionary response to the risk posed to the species by herbivores, many of them are insects. Hence this species shows insecticidal activity. Near about 2000 plant species are reported to have compounds with pest control properties. Nicotine, derris and pyrethrum are some of the more efficient compounds have long been exploited commercially as household and agricultural insecticides. Apart from these many others also used traditionally in

Africa but their use is hardly ever general with particular plants being used as insect repellents in one area but disused in another area.² Even though the usefulness of extracts from most of these plants is unfortunately compared to profitable synthetic pesticides, still they can supply significantly to pest control and pest management when their use is incorporated with another measures and frequently have the benefit of low toxicity to human. Some plant extracts are active against insect pests, fungal and bacterial pathogens. *Datura stramonium* has been shown to give control of both *Alternaria* leaf spot (*Alternaria macrospora*) and bacterial blight (*Xanthomonas campestris* pv. *malvacearum*) on cotton.³

Family: Asteraceae

In the Angiosperm phylogeny the Asteraceae family is nested high in Asterideae/Asterales. There are 1600–1700 genera dispersed around the world except in Antarctica, assume that there are 2,50,000–3,50,000 species of flowering plants, then one out of every eight to twelve species is of Asteraceae means nearly about 10% flowering plants is of this family. Hence it proves that Asteraceae is monophyletic family. Nowadays every worker in plant classification familiar Asteraceae as a group at some level and in each type of examination the family is monophyletic. The family is characterizes by florets arranged on a receptacle in centripetally developing heads and enclosed by bracts, by anthers merged in a ring with the pollen pressed or brushed away by the style, and by the presence of achenes or cypselas usually with a pappus. Though the family is clear, there is a large deal of deviation among the members like the habit vary from annual to perennial herbs or shrubs,



vines, or trees, even though few are accurately epiphytes then species grow in every type of habitat from forests to high elevation grasslands, on the other hand, they are less common in tropical wet forests but more common in open areas. Most groups in the family contain some functional and some deadly poisonous species as well as common and exceptional taxa. However, the common observation of this family as it contains only weeds or as a “weedy” is not right. Surely there are members that profitable from disturbance, like a few species of dandelions as well as thistles, and a only some global pests like e.g., *Chromolaena odorata* (L.) R.M. King & H. Rob. Most species have a limited distribution and about each ‘at risk’ habitat in the earth contains members of this family that are an significant part of the flora.⁴

Description of family frequently annual herbs, biennial or perennial herbs, as well as subshrubs, and shrubs, vines or trees, polycarpic or monocarpic, pachycaul or leptocaul usually terrestrial and rarely epiphytic or aquatic, occasionally succulent, generally with various types of glandular or eglandular hairs, usually the eglandular uniseriate and glandular biseriate. Tissues typically with schizogenous secretory canals or resin-ducts and with articulated laticifers. Alternate or opposite Leaves, rarely whorled, typically simple but often lobed or may be divided exstipulate. Unit of inflorescence a capitulum or head, and rare exceptions bounded by an involucre of one to several sequence of protective bracts or phyllaries, capitula may be solitary at the apex of more or less leafless stems also called scapes but many in often *Corymbiform cymose* inflorescences is capitulescences, synflorescence of a variety of types, now and then aggregate into frequently involucre capituliform syncephalia of the second or even third order. Receptacle are either naked or may be hairy and smooth, as well as areolate with polygonal or many-sidal areoles also known as alveolate with depressions where the florets are inserted, or paleate with persistent or caducous vascularized scales like paleae, pales, chaff, among all the florets, and also base of the florets is surrounded by scale like processes hence they known to be fimbriiferous with non-vascularized fimbriils.⁴

Plants from Asteraceae family are spread throughout the world and most common in the arid and semi-arid regions of subtropical and lower temperate latitudes. These plants normally have hairy and strong aromatic and fragrant leaves and flat clusters or bunches of small flowers at the top of the stem. Because of various attractive colours of flowers, a number of species from this family are known as popular garden plants. The greater part of the Asteraceae family members are as the medicinal plants which have beneficial and remedial applications.

***Blumea lacera*: As A Weed**

Plants which are used for estimation of essential and heavy metals are from the Asteraceae family, locally they are used as medicinal herbs but commonly found in local

region as a weed. *Blumea lacera* is described as a valuable medicinal plant in many vital systems of medicines including Ayurveda homeopathy and Yunani or unani. *Blumea lacera* is an erect villous herb. 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. It shows great antimicrobial activities as well as its essential oil also shows some medicinal properties hence this weed used as medicinal plant.⁵

***Cyathocline purpurea*: As A Weed**

Cyathocline purpurea is an annual and occasionally perennial. Flowers are usually purple in colour and occur in corymbs at the end of branches. This weed plant is also shows great importance in medicinal field mostly as an anti-inflammatory agent so it is also a medicinal plant species.⁶ *Cyathocline* species are active as a medicinally important plants.⁷

The plant used in medicines, the roots are used in treating stomach pain. 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. They are found in elemental form and in a variety of other chemical compounds. A heavy metal, depending on the context, is usually regarded as a metal or sometimes a metalloid with high density and atomic weight or with atomic number, is often assumed to be toxic metal. Some heavy metals, such as cadmium, chromium, mercury, arsenic and lead are highly toxic.⁸

While others are essential nutrients in trace amounts or are relatively harmless. Some essential minerals like iron, zinc, copper, cobalt, sodium and manganese are needed for proper physiological function while they present in low amount; however their higher concentrations of these metals can be toxic.⁹ There are other metals such as mercury, lead that are toxic to living things.¹⁰ Some heavy metals given below shows their presence in result of chemical analysis. There are total 20 elements were analyzed out of that 13 are essential nutrients while others are toxic heavy metals but essential nutrients also shows adverse effects on plant growth metabolism like nickel as well as some enzymatic and biochemical reactions occur within cells when they are present in excess amount.^{11,12}

Biologically active nature of *Blumea lacera* and *Cyathocline purpurea*

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.¹³ *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 contain 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.¹⁴

Blumea lacera has significant medicinal uses. Methanolic extract of *Blumea lacera* shows better result for biological activities like cytotoxic nature capacity, antifungal activity, antibacterial activity, antipyretic activity, antiviral and antidiarrhoeal activity.¹⁵ Traditional use of *Blumea lacera* is as a antifungal agent, antiviral active component, antihelmintic as well as to cure dysentery also.¹⁶ *Cyathocline purpurea* extract also shows biological activities like analgesic activity (peripheral analgesic effect), anti-inflammatory activity, and antioxidant activity by using H₂O₂ radical scavenging assay method. These activities have been observed on rodents. Hence plant extract has many medicinal uses on living beings.¹⁷ 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.¹⁸

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.

MATERIALS AND METHODS

Collection of sample plants

Plant samples were selected for investigation are *Blumea lacera* and *Cyathocline purpurea*. (Dr. V. B. Shimpale, Department of Botany, Shivaji University, Kolhapur). Plant sample collected from local area (Kolhapur region). After

collecting fresh plant samples, they get separated from dirt and adhered soil as well as followed by washing and cleaning under water flow to remove attached dust. And fresh weed sample were used for oil extraction process.

Antibacterial activity

a. Microorganisms

The Gram negative bacteria i.e. *Pseudomonas aeruginosa* (NCIM 5032), and Gram positive bacterial species i.e. *Bacillus subtilis* (NCIM 2635), were used to perform antibacterial activity. Bacteria strains were obtained from Department of Microbiology, Shivaji University, Kolhapur. Bacteria were maintained on nutrient agar slant and Sabouraud's Dextrose Agar (SDA) respectively then stored at 4°C. Bacteria were sub-cultured onto fresh media at regular intervals until used.

b. Antibacterial activity screening

The first screening step, in this study, was carried out to prop the antibacterial activity of aqueous concentrations of an essential oil of weed species i.e. *Blumea lacera* and *Cyathocline purpurea*, against *Bacillus subtilis* (NCIM 2635) and *Pseudomonas aeruginosa* (NCIM 5032), as a gram positive and gram negative bacterial species respectively. All experiments were duplicated. The diameter in mm of the clear zone indicated the inhibition activity. In the screening, the antibacterial activity of the concentrations was done by 'Agar Well Diffusion Assay.' The diameters of inhibition zones were measured in mm after incubation at 37°C for 24 h. Each sensitivity test was performed by using an antibiotic drug as a control. The antimicrobial activity was measured by the inhibition zones produced. All the tests were performed in duplicate. The antimicrobial activities of the selected plant extracts against the tested bacteria were compared with the available antibiotics. The plates were incubated after incubation the diameter of the inhibition zones were measured in mm and recorded.¹⁹

RESULT AND DISCUSSION

Biological Activities

Biologically active nature of *Blumea lacera* and *Cyathocline purpurea*

Blumea lacera were identified as medicinally important against headache, it is an aromatic herbal species, and also it shows anticancer activity.²⁰ *Blumea lacera* in solvents like methanol and petroleum ether shows presence of phytochemicals, in earlier investigation it was found that it has antidyseric as well as antidiarrhoeal properties because it contains phytochemicals. This species shows good cytotoxic nature and Antimicrobial activity.²¹ *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.²² It shows essential bioactive constituents and antimicrobial activity hence it



is a valuable plant with reference to their medicinal values. 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.²⁴

Antibacterial activity results

Essential oil was extracted from *Blumea lacera* and *Cyathocline purpurea* by using Clevenger apparatus. Fresh extracted oil was studied for their antibacterial activity. one bacterial species from agricultural field i.e. *Pseudomonas aeruginosa* (NCIM 5032) and one bacteria as a human pathogen i.e. *Bacillus subtilis* (NCIM 2635) were studied for this activity. Inhibition zone was measured in mm, and recorded in Table 1 and 2 respectively. Essential oil of *Blumea lacera* inhibits growth of *Bacillus subtilis* at concentration 100 μ l. while it inhibits growth of *Pseudomonas aeruginosa* at concentration 75 μ l. On the other hand essential oil of *Cyathocline purpurea* inhibits growth of *Bacillus subtilis* at very low concentration i.e. 25 μ l. while it inhibits growth of *Pseudomonas aeruginosa* at concentration 50 μ l. Oil extracted from *Cyathocline purpurea* shows better antibacterial activity at lowest concentration.

Essential oil was extracted from *Blumea lacera* and *Cyathocline purpurea* shows very good antibacterial activity and growth inhibition zone at lowest concentration as shown in Fig. 1 and Fig. 2 respectively. From these observations we can say that both selected weed species will prove to show good agricultural applications, in case of inhibition of bacterial growth. Antibacterial activity results for essential oil extracted from *Blumea lacera* are given in Table 1 While Table 2 shows Antibacterial activity results for essential oil extracted from *Cyathocline purpurea*. Essential oil from *Blumea lacera* in concentration 100 μ l shows inhibition zone of 1.5mm for *Bacillus subtilis* (NCIM 2635). While 75 μ l shows inhibition zone of 1.5mm for *Pseudomonas aeruginosa* (NCIM 5032). Essential oil from *Cyathocline purpurea* in concentration 25 μ l shows inhibition zone of 1.1mm for *Bacillus subtilis* (NCIM 2635). While 50 μ l shows inhibition zone of 1.3 mm for *Pseudomonas aeruginosa* (NCIM 5032).

Table 1: Antibacterial activity results for essential oil extracted from *Blumea lacera*

Bacteria	Concentration in μ l			
	25	50	75	100
<i>Bacillus subtilis</i> (NCIM 2635)	-	-	-	1.5mm
<i>Pseudomonas aeruginosa</i> (NCIM 5032)	-	-	1.5mm	1.7mm

(Zone of bacterial growth inhibition is given in mm)

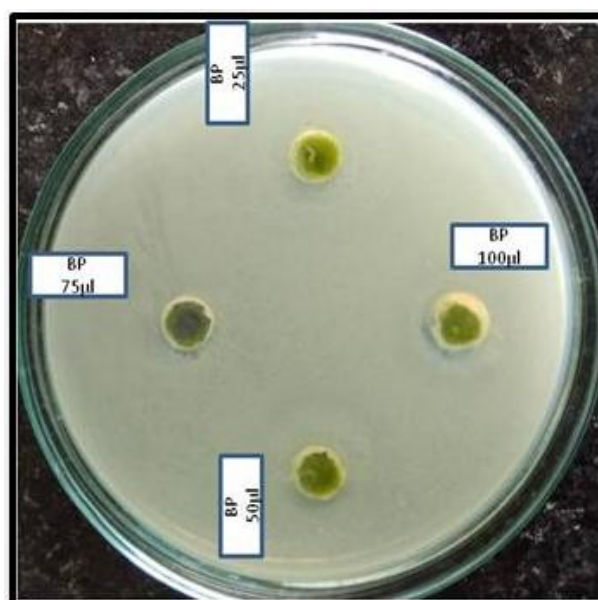
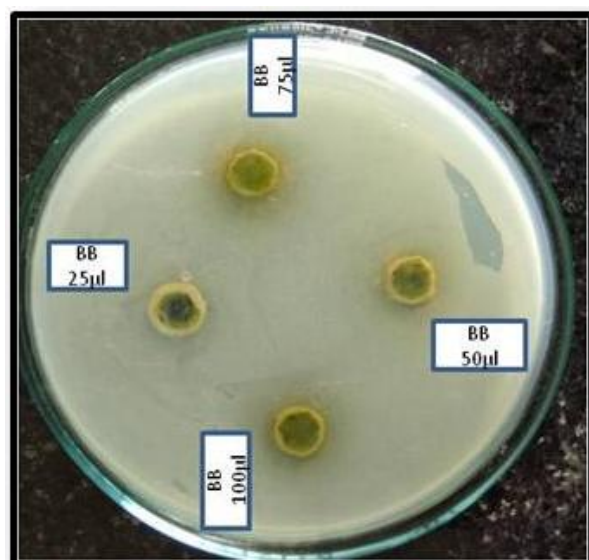


Figure 1: Antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* for essential oil extracted from *Blumea lacera*.

Table 2: Antibacterial activity results for essential oil extracted from *Cyathocline purpurea*

Bacteria	Concentration in μ l			
	25	50	75	100
<i>Bacillus subtilis</i> (NCIM 2635)	1.1mm	1.2mm	1.7mm	1.8mm
<i>Pseudomonas aeruginosa</i> (NCIM 5032)	-	1.3mm	1.3mm	1.5mm

(Zone of bacterial growth inhibition is given in mm)

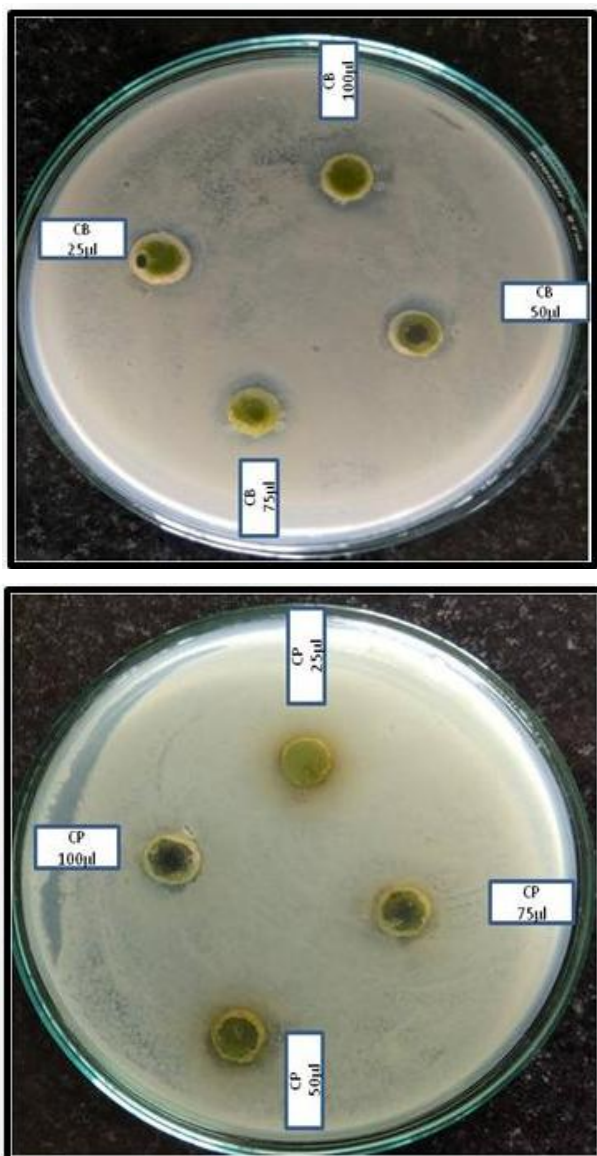


Figure 2: Antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* for essential oil extracted from *Cyathocline purpurea*

CONCLUSION

Extracted essential oil from *Blumea lacera* and *Cyathocline purpurea* were studied for antibacterial activity. Two bacterial species such as *Bacillus subtilis* and *Pseudomonas aeruginosa* are studied and their growth is inhibited due to active nature of extracted oil. Better activity proves that oil from weed species shows good agricultural applications regarding bacterial growth. Hence, essential oil has also pharmacological properties.

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