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Screening on antioxidant activity, antimicrobial activity and phytoconstitutents of *Cythocline lyrata* leaf

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ABSTRACT

The present study focuses on the phytochemical analysis, Antioxidant activity and Antimicrobial activity of *Cyathocline lyrata* leaf collected from Melghat Forest Tal- Chikhaldara, Dist- Amravati. Phytochemical screening was carried out by 'guide to modern techniques of plant analysis". Furthermore antioxidant activity of methanolic extract of *C. lyrata* was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Antimicrobial activity of *C. lyrata* was investigated by Agar disc diffusion assay. The Phytochemical screening of aqueous, methanolic and Chloroform leaf extracts revealed the presence of various secondary metabolites. The FTIR analysis of the crude extract of *C. lyrata* gives information about the distribution of functional groups and provides a basis for comparison of compositional differences between isolates and among samples. The DPPH radical scavenging activity of *Cyathocline lyrata* extract with ascorbic acid as reference, where the IC_{50} values for the *Cyathocline lyrata* leaf extract (IC_{50}= 2047 µg/ml) and IC_{50} value were said to be less than the standard ascorbic acid (IC_{50}= 2.816 µg/ml). *Cyathocline lyrata* ethyl acetate leaf extract showed good inhibition against all six Organisms. The highest inhibition was noted P.aeru (11 mm), P.acne (11 mm), K. pneumonia (10 mm). This study revealed the presence of phytochemical compounds and presence of functional groups. The study concluded that the methanolic extracts shows significant antioxidant activities in a concentration dependant manner and also shows significant antibacterial activity hence the plant contains potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments

Keywords: Phytochemical, FTIR, Antioxidant, Antimicrobial

1. INTRODUCTION

The plant *Cyathocline lyrata* belongs to family Asteraceae. It is commonly called as Jungli kante and sevati. It is a slender delicate annual herb; growing to 20-25 cm high, branched grooved stem has soft hair covering it. Whole plant is strongly aromatic. Alternatively arrange stalk less leave are toothed covered with soft hair and flowers occurs in corymbs at the end of branched in rose-purple color. *Cyathocline lyrata* widely spread in Himalayas range, Assam, India (Local area of Maharashtra). Usually met with on the banks of streams and in moist localities. Worth growing in gardens in moist regions a native of India. [1] Cyathocline lyrata is well known drug in Indigenous system of medicine for its various used as a bitter tonic. It acts as germicide and appetizer. The essential oil of aerial part of *Cyathocline lyrata* had show fairly pharmacological activity. It also shows antioxidant, insect repellant and antimicrobial activity.[2] C. lyrata is used for isolation of essential oil and alcohol. Biological screen includes tests for antibacterial, antiprotozoal, antiviral antifungal and pharmacological activities.[3]

2. MATERIALS AND METHODS

2.1. Collection of Sample

Fresh Leaves of *Cyathocline lyrata* were collected from Melghat Forest Tal- Chikhaldara, Dist- Amravati (Central region of India) in the month of February –2013 and plant authenticated by a taxonomist from Department of Botany ACS College Amravati .

2.2. Processing of the sample

Fresh Leaves of plants were washed well using tap water and twice using distilled water and it was dried in shade for a period of 10-12 days, at an ambient temperature of 30˚C. After drying plant leaves were cutting into small pieces. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form and stored at room temperature till their use in the experiment.

2.3 Preparation of extracts

Dried powdered material (20 gm) of sample was extracted with methanol, distilled water and chloroform separately in soxhlet apparatus. The temperature of heating mantle was adjusted to 60ºC for methanolic extraction while 100ºC for aqueous extraction and 55ºC for chloroform extraction. The extracts were concentrated by gradually evaporating the respective solvent on hot water bath. The concentrated extract was collected in sterile bottles and refrigerated until use. [4]

2.4. Preliminary phytochemical investigation

The major secondary metabolites classes such as alkaloids, Carbohydrates, Amino Acid, Reducing Sugar, Tannins and Phenolic Compound, Saponins, Flavonoids, Cumarine and Terpenoids were screened according to the common phytochemical methods described by A guide to modern techniques of plant analysis, Medicinal Plants and Traditional Medicine in Africa and Pharmacognosy [5-7] (Table 1).

2.5. FT-IR Analysis of crude powder of Cyathocline lyrata

FT-IR is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of leaves of *Cyathocline lyrata* plant materials was considered for instrumental analysis. The powdered sample plant specimens were treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). Scan range: from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} [8, 9]

2.6. Antioxidant activity of cyathocline lyrata

2.6.1. Sample Preparation

The concentrated methanolic extract was transfer to various Petri dishes for evaporating the methanol. Evaporating is done in ambient temperature and dried powder collected in sterilized eppendorf tube and stored at 4 ^{0}C for further use.

The stock solution of crude extracts was prepared by dissolving a known amount of dry extract in 98% methanol. The working solutions are (1000, 2000,3000,4000,5000 µg/ml) of the extracts were prepared from the stock solution using suitable dilution. [10]

2.6.2. DPPH free radical scavenging activity of methanolic extract

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2, 2-diphenyl-1- picrylhydrazyl (DPPH) free radical was determined by the method described by 'Fully Automated Spectrometric Protocols for Determination of Antioxidant Activity: Advantages and Disadvantages",[11]

The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1000-5000 µg/ml solution. 3.94 mg of DPPH was prepared in 100 ml methanol and 2.96 ml of this solution was mixed with 40 µl of sample solution and standard solution separately. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using UV-Vis Spectrophotometer (UV-1700 Shimadzu). DPPH solution was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below

% of DPPH radical scavenging activity (%RSA) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

$Abs_{control\ is}$ the absorbance of DPPH radical + methanol: Abs_{sample} is the absorbance of DPPH radical + Sample extract .the measurements were performed in triplicate. Absorbance values were corrected for free radical decay using blank solutions. The IC_{50} (Concentration providing 50% inhibition) was calculated graphically using calibration curve verses' percentage of inhibition.[12]

2.7. Antimicrobial activity of cyathocline lyrata

2.7.1 Sample Preparation:

The concentrated methanolic extract was transfer to various Petri dishes for evaporating the methanol. Evaporating is done in ambient temperature and dried powder is mixed with ethyl acetate solution. These ethyl acetate plants extract further use for antibacterial activity.[13]

2.7.2 Test Bacteria

Escherichia coli (ATCC-14948), Staphylococcus aureus (ATCC-33591), Klebsiella pneumonia (MTCC-4030), Pseudomonas aeruginosa (ATCC-4676), Propionibacterium acnes (ATCC-1951), Salmonella typhi (ATCC-25812),were purchased from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh,

India and used for assessment of antibacterial activity.

Table – 1: Phytochemical analysis of *Cyathocline lyrata* leaf

Phytochemical	Tests performed	Aqueous extract	Methanolic extract	Chloroform extract
Carbohydrates	Molish Test	++	+	+
Amino Acid	Ninhydrin Test	++	-	-
Reducing Suger	Fehling Test	++	-	++
	Benedict Test	++	-	++
Protein	Biuret Test	-	-	+
	Xanthoprotic Test	+	++	-
Steroid	Ring Test	+	++	++
Phytosterols	Libermann Buchard Test	-	-	+
Tannins and Phenolic Compound	Ferric Chloride Test	-	-	-
	Lead Acetate Test	++	+	+
	Gelatin Test	-	-	-
Anthranol Glycosides	Borntrager's Test	+	-	-
Cardiac Glycosides	Legal Test	-	+	++
Terpenoids	Salkowski Test	+	+	+
Alkaloids	Mayer Test	-	-	-
	Dragendroff's Test	-	-	-
	Wagner Test	+	+	+
	Ethyl acetate Test	-	+	++
Flavonoids	Alkaline reagent Test	+	-	++
Cumarine	Fluorescence test	+	++	+
Saponins	Foam Test	+	-	-
fixed oils and lipids	Spot Test	-	-	-

indicates: strong presence, + indicates: weak presence, - indicates: strong absence

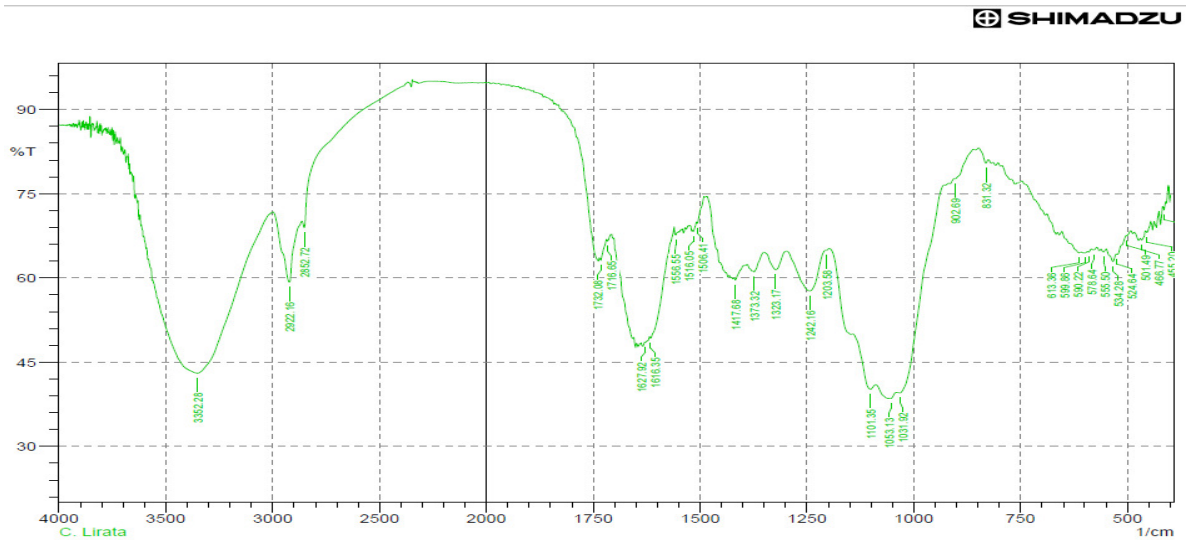


Figure - 1: FT-IR for *Cyathocline lyrata* leaf

2.7.3 Antibacterial screening

Agar disc diffusion assay according to the Manual of antimicrobial susceptibility testing [14] was used to assay the various antibiotics for bactericidal activity against test strains of E. coli, K. pneumonia, P. aeruginosa, S. aureus, P. acnes, S.typhi. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37 ^{0}C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37 ^{0}C Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Rifampicin (10 µg/disc) was used as standards.

3. RESULTS AND DISCUSION

The result obtained in the present investigation (Table 1), the aqueous, methanol and chloroform extracts of the powdered leaves of *Cyathocline lyrata* showed the presence of following phytoconstituents. aqueous extract of *Cyathocline lyrata* revealed the presence of carbohydrates ,amino acids, reducing sugar, tannins ,phenolic compound ,flavonoids, Saponins, coumarines, steroids. methanolic extract of *Cyathocline lyrata* revealed the presence of protein ,steroids, coumarines, tannins, phenolic compound, Saponins, Alkaloids, cardiac glycosides, terpenoids Chloroform extract of *Cyathocline lyrata* revealed the presence of reducing sugar ,steroids, cardiac glycosides , Alkaloids , tannins and phenolic compound, terpenoids, These Phytochemicals may be responsible for their insecticidal properties. [15] The presence of tannins shows that the plants can be used as purgative. They are also used in the treatment of cough, asthma and hay fever.[16] The presence of terpenoids revealed that the plants can act mainly as anti-feedant and growth disruptor and possesses considerable toxicity toward insects. [17]

Terpenoids also plays an important role in wound and scar healing.[18]It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones. The presence of steroidal compounds in the plants is an indication that the plants can be used or expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones. [19]

Cyathocline lyrata leaf, FTIR- Spectrum (Figure 1) shows strong absorption peaks at 3352.28cm^{-1}which shows strong absorbency N-H Stretch for aliphatic secondary amine, peaks at 2922.16 and 2852.72 cm^{-1}represents identical absorbency of symmetrical stretching frequency for methelene C-H it indicates aliphatic hydrocarbon,

The peaks at 1732.08 cm^{-1}stretching frequency of CHO it indicates aldehydic group and 1716.65 cm^{-1}stretching frequency of COOH it indicates carboxylic group, The peaks at 1627.92 cm^{-1}stretching frequency of CO-NH it indicates amide group . The peaks at1516.05 and 1506.41 cm^{-1}represents identical absorbency NO_2 indicate presence of aromatic nitro group. The peaks at1373.32cm^{-1} indicate aliphatic nitro group and peaks at 1323.17cm^{-1} indicate presence of aromatic nitro Compound. The peaks at 1242.16 and 1203.58 cm^{-1}represents identical absorbency of stretching frequency of (P-O-C) stretch it indicates aromatic phosphate. The peaks at1053.13 and 1031.92 cm^{-1}represents identical absorbency of stretching frequency of Si-O-Si it indicates silicone or organic siloxane. The peaks at 902.69 indicate presence of silicate ion and peak at 831.32 shows presence of nitrate ion NO_3. The peaks at 613.36 indicate presence of disulfide S-S stretch. [20]

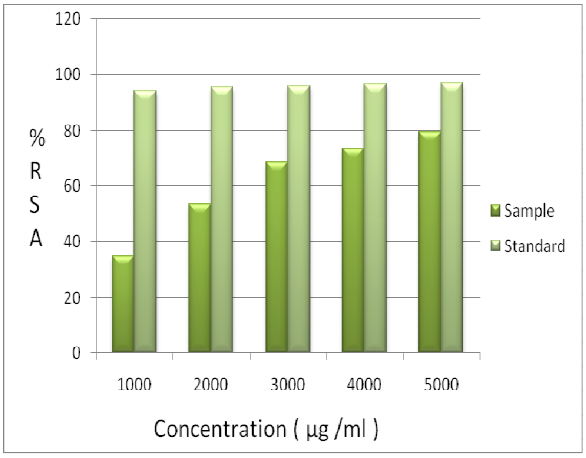


Figure - 2: DPPH radical scavenging activity of *Cyathocline lyrata* leaf extract

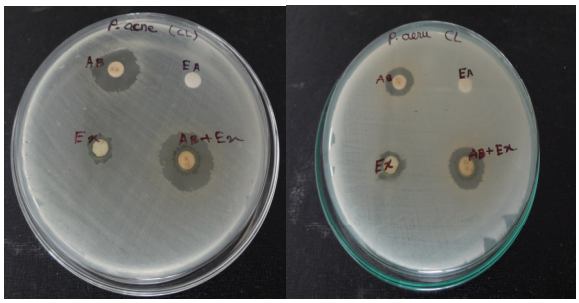
The radical scavenging activity of the *Cyathocline lyrata* methanolic leaf extract was tested using stable free radical DPPH (deep purple colour), as DPPH has the advantage of being unaffected by certain side reactions. Figure 2 shows the DPPH radical scavenging activity of *Cyathocline lyrata* extract with ascorbic acid as reference, where the IC_{50} values for the *Cyathocline lyrata* leaf extract (IC_{50}= 2047 µg/ml)

Table - 2: Antibacterial activity of *Cyathocline lyrata* Ethyl acetate extract

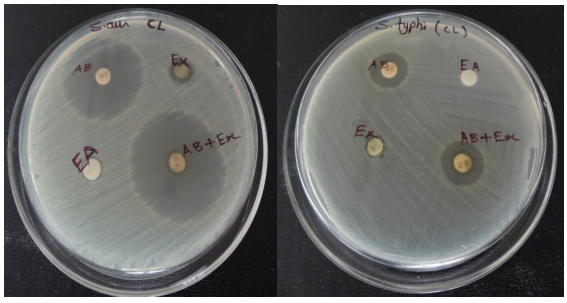
Test Organism used	Extract	Antibiotic (Rifampicin)	Antibiotic + Extract	Ethyl acetate (Control)
K.pneu	10	18	19	0
P.aeru	11	13	13	0
S.aur	10	34	34	0
E.coli	10	18	19	0
P.acne	11	19	20	0
S.Typhi	09	15	15	0
Zone of inhibition in Diameter (mm)				

and IC_{50} value were said to be less than the standard ascorbic acid (IC_{50}= 2.816 µg/ml). The presence of flavonoids and tannins in the plants in phytoconstituent responsible for the free radical scavenging effects. Flavonoids and tannins are the phenolic compounds and plants phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [21]

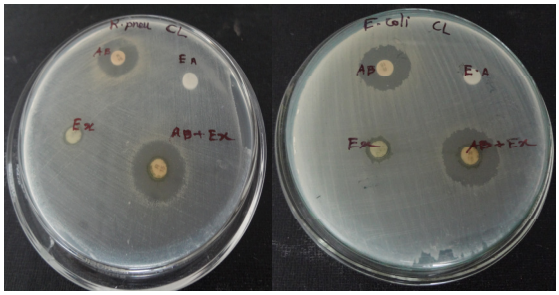
Cyathocline lyrata ethyl acetate leaf extract showed good inhibition against all six Organisms (Table 2 and Figure -3). The highest inhibition was noted P.aeru (11 mm), P.acne (11 mm), K. pneumonia (10mm). The ethyl acetate extract of *Cyathocline lyrata*, possessed significant antioxidant activity and antimicrobial activity due to the presence of various phenolic compounds. [22]



P-aeru (CL) **P-acne (CL)**



S-aur (CL) **S-typhi (CL)**



K- pneu (CL) **E-Coli (CL)**

Figure - 3: Antibacterial activity of *Cyathocline lyrata* Leaf against six bacteria, in each image: AB- Antibiotic disk, E.A- Sterile disk (control), EX- Extract disk, AB+ EX- Antibiotic + Extract disk.

4. CONCLUSION

Cyathocline lyrata has been subjected to preliminary screening of phytoconstituents and FTIR. This study revealed the presence of phytochemical compounds and presence of functional groups of compounds such as Secondary amine, aldehydic, carboxylic, disulfide, Ar- nitro, Ar- phosphate, silicone or organic siloxane, amide group . The study concluded that the methanolic extracts shows significant antioxidant activities in a concentration dependant manner and also shows significant antibacterial activity hence the plant contains potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments and also plant extracts can be used for the treatment of infections caused by the strains of the test bacterial organisms.

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