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Isolation of Plant Extract (Hydroalcoholic Extract), Phytochemical Investigation, Total Phenol and Flavanoid Activity and Antioxidant Activity of *Cythocline purpurea*

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ABSTRACT

Present study deals the study of *Cyathocline purpurea* species of family Asteraceae The aim of the present study was to identify Isolated extract different types of extraction process, Identify chemical constituents with the help of phytochemical screening, total phenol & flavonoid activity, antioxidant activity,& antibacterial activity.

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INTRODUCTION-

This plant is isolated from Chittorgarh district Rajasthan system of medicine for its various used as a bitter tonic. It acts as a germicide and appetizer and stomach pain. Essential oils are volatile chemical products formed by odoriferous substances isolated from great variety of plant. These are water insoluble but highly soluble in vegetables and minerals oil and ether. They are categorized according to their main volatile components. *Cyathocline purpurea* is indigenously found near Lonavala in Maharashtra. Aerial part of the plant used for the isolation of essential oil and alcohol. Biological screen includes tests for antibacterial, antiprotozoal, antimicrobial, antioxidant anti fertility and pharmacological activity. The pharmacological properties of aromatic plants are partially attributed to essential oils. Essential oil are natural, complex, multi-component systems composed mainly of terpenes. Plant continue to serve as possible sources for drugs and chemicals derived from medicinal plants have proven to be an abundant source of biologically active compounds many have been the basis for the development of new lead chemicals for pharmaceuticals.

OBJECTIVE OF THE STUDY-

1) Collection of *Cyathocline lyrata* Kuntz, family Asteraceae of his ethnobotanical important.

2) To study the phytochemicals of hydroalcoholic leaf extract of *Cyathocline lyrata*.

3) Quantitative analysis of phenol and flavonoid of plant leaf extract by spectrophotometric method.

4) To test the antioxidant efficacy of the leaf extract with reference to standard values.

Cyathocline lyrata is annual and occasionally perennial. Flowers is usually purple in color and occurs in corymbs at the end of branches. This weed plant is also shows great importance in medicinal field mostly as anti inflammatory agents so it is also medicinal plant species cyathocline species are active as medicinally important plants.

The plant used in medicine the root is used in treating stomach pain. Some heavy metals are analyzed from these two plant sample for the purpose of studying the presence of their quantitative factor. Heavy metals are present in varying concentrations in different plants. They are found in elemental form and in a variety of other chemical compounds. A heavy metals depending on the context is usually regarded as 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.

FAMILY: Asteraceae.

In the angiosperm phylogeny, the Asteraceae family is nested high in Asteraceae.

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 8-12 species of Asteraceae means nearly about 10% flowering plant is of his family. Nowadays every worker in plant classification familiar Asteraceae as a group at some level and each type of examination the family is monophyletic.

MATERIALS AND METHODS

Fresh plant of *Cyathocline lyrata* were dried at room temp. (25 to 30) for 7 days. Whole plant of *Cyathocline lyrata* was collected from local area of M.P (campus of Vikram University Ujjain) authenticated by Dr. Pathak.

1) Collection of plant material-

Fresh plant of *Cythocline lyrata* were dried at room temp.(25 to 30) for 7 days. Whole plant of *Cythocline lyrata* was collected from local area of M.P (campus of Vikram University Ujjain).

2) Authentication of plant material-

The plant material were authenticated by the department of botany, MLV Govt College, Bhilwara, Rajsthan and the voucher specimens were deposited their under accession no.1295.

Extraction- The fresh leaves of *Cythocline lyrata* were washed with distilled water and shade dried at room temperature and pulverized into coarsely powder in a laboratory grinder 100 gm of dried leaves powder was extracted with hydroalcoholic solvent with maceration

process. The plant with hydroalcoholic solvent was kept at 24hrs.to obtain the extract after that extract was evaporated in water bath at 55 obtain crude as semi solid mass.

3) Qualitative analysis

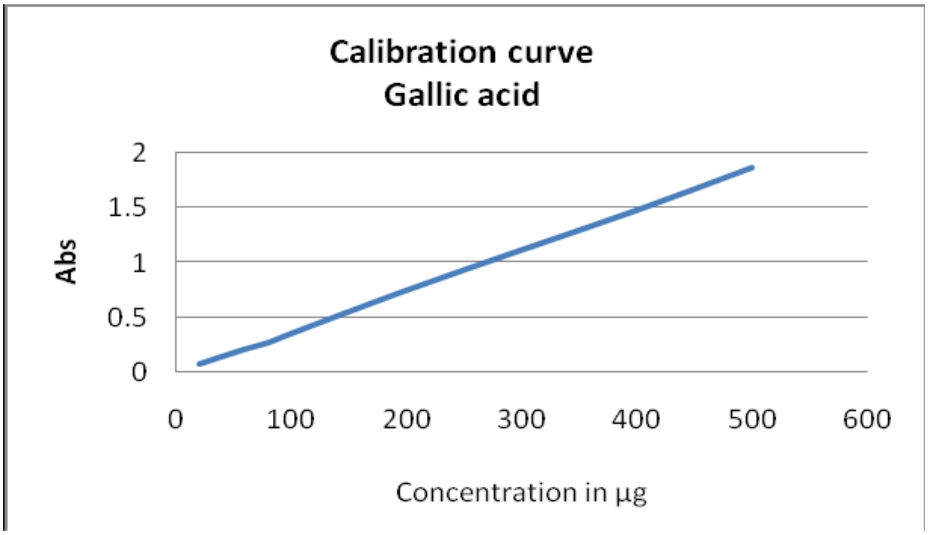
NO. Test	Pet. Ether	Ethanol	Benzene	Chloroform	Water
1.Carbohydrate Fehling test	-	-	-	-	-
2.Protein Xanthoproetin test	+	+	-	+	-
3. Alkaloid- Mayers test	+	-	-	+	
4. Phenol test $Fecl_3$test	+	+	+	+	+
5.Amino Acid Ninhydrin test	+	-	-	+	
6. Steroid Salkowski test	-	+	-	+	
7.Flavonoid Alkali reagent test	+	+	+	+	
8. Glycoside Killer Killani test	+	-	-	+	
9. Saponin test Foam test	+	-	+	-	

4) Total Phenol content-

We have Perform the total phenol content activity by using phenol catechu method 2ml of extract of that plant 1ml of FC reagent (1:10) was added 1 ml of sodium carbonate kept for 20 min. for incubation period and taken absorbance at 765nm by using spectrophotometry.

Total phenolic content of hydroalcoholic extract in mg/equiv.to gallic acid

Sr. No.	Absorbance of extract	Concentration of extract	Total phenolic content mg/g equiv. to Gallic Acid
1	0.352	1mg/ml	28.0
2	0.354	1mg/ml	30.0
3	0.350	1mg/ml	29.0
	Mean ± SD		28±.05



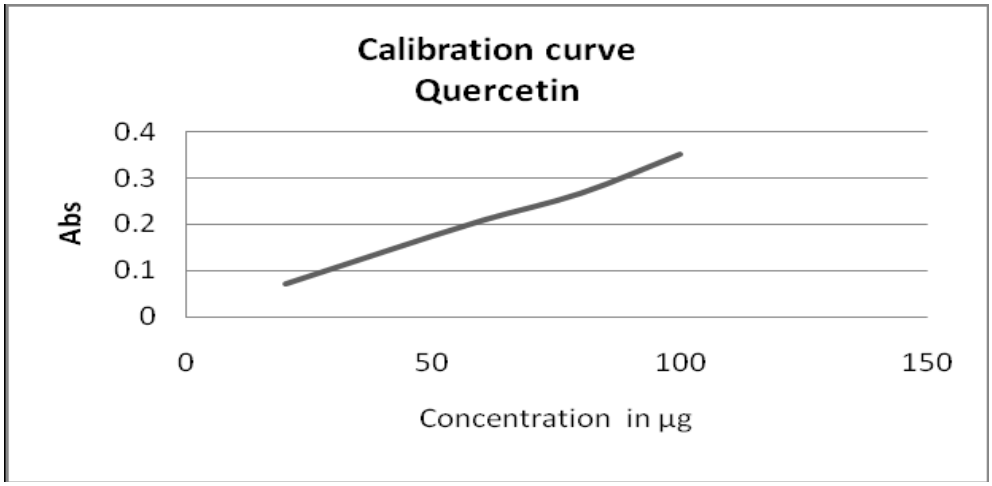
Calibration curve of (gallic)

5) Total flavonoid content-

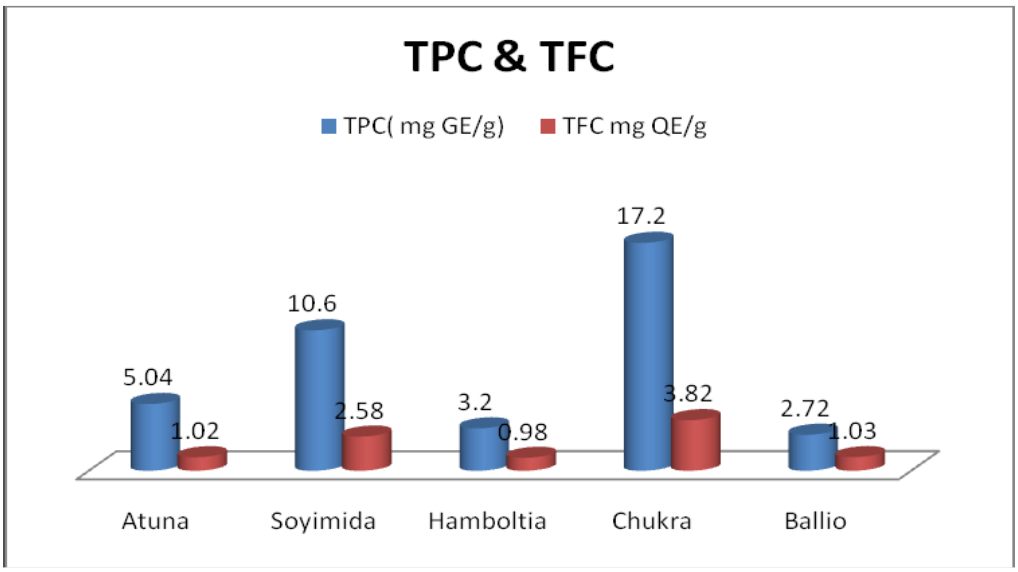
We have perform the total flavonoid content activity by using $Alcl_3$ method 3ml of extract was taken 1ml of $Alcl_3$ was added kept 10min for incubation period and taken absorbance at 420 nm by using spectrophotometer.

Total flavonoid content of hydroalcoholic extract in mg/equiv. to quercetin

Sr. No.	Absorbance of extract	Concentration of extract	Total flavonoid content mg/g equiv. to Gallic quercetin
1	0123	1mg/ml	09
2	0.124	1mg/ml	8.5
3	0.122	1mg/ml	08
	Mean ± SD		8.5±05



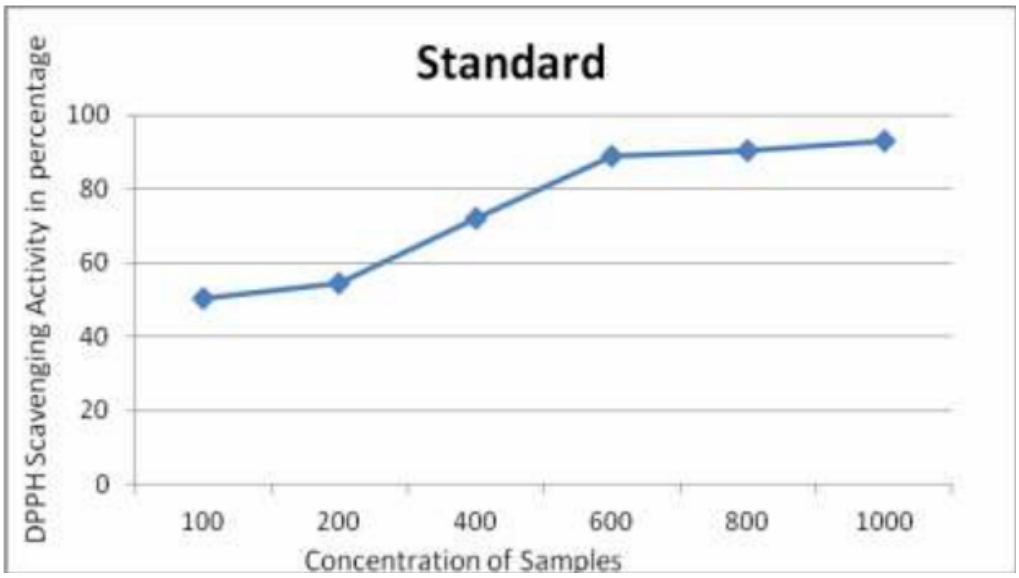
Calibration curve of quercetin



Total phenol and flavonoid content of the *Cyathocline lyrata*

6) Antioxidant activity by using DPPH method

A) DPPH method: 1.5ml of extract was taken and 1.5 ml of DPPH solution kept for 15 min.for incubation period and taken absorbance at 490nm by using spectrophotometer.



B) Total Radical-Trapping Antioxidant parameter (TRAP) method: This method used a luminescence spectrometer to measure the fluorescence decay of R-phycoerythrin during a controlled peroxidation reaction. The TRAP value are calculated from the length of the lag-phase caused by the antioxidant as compare to that of Trolox.

C) Ferric reducing/antioxidant power (FRAP) method:

This method measure the ability of antioxidant to reduce ferric ion. It is based on the reduction of the complex of ferric ion to the ferric ion at low pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer.

7) Thin layer chromatography:

TLC of successive solvent extract of whole plant of *Cyathocline lyrata* TLC was an important tool in the separation, identification and estimation of different compounds, Here, when we spot a mixture of components, which were readily soluble, but not strongly adsorbed moves up along with the solvent and those not so soluble but more strongly adsorbed moves up less readily to separation of compound. TLC was performed on the precoated TLC aluminum sheets material silica gel the TLC chamber was saturated with the solvent and after that applying the spot on TLC plates were kept for development of chromatogram. Then the separation were visualized by the detecting reagent were studied.

Procedure:

Approximately 10 microlitres of sample solution was applied on a precoated silica gel aluminium plates of uniform thickness. The plate was developed in the solvent up to a distance of 10 cm.

Visualization:

The plate was observed under UV at 254 and 366 nm and then exposed to iodine vapors. The plate was also sprayed vanillin-sulphuric acid reagent heating plate for 10min at 110 degree.

Formula:

RF= distance travel by solute/distance travel by solvent

Detail of solvent system for TLC

Extract	Solvent system	Spray reagent	No.of spots	Rf.value
Pet.Ether	Hexane, Ethyl acetate	Exposed to I_2 vapour	02	0.25,0.15
Benzene Toluene,	Ethyformate Formic acid	Exposed to I_2 vapour	02	0.21,0.33
Chloroform	Benzene, Ethyl Acetate	Exposed to I_2 vapour	04	0.23,0.24
Ethanol	Butanol Acetic Acid, Water	Exposed to I_2 vapour	02	0.22,0.35
Water Butanol,	Acetic Acid, Water	Exposed to I_2 vapour	06	0.16,0.22

8) Antimicrobial activity

Isolation of Dental bacterial strains

Antimicrobial activity, 20 samples from different dental microflora of patients from Bishnoi dental hospital nimbahera were isolated by using swab method and spread on nutrient agar media plates and then were incubated at 37. After culturing the bacterial isolates were stained by Gram's staining and observed under compound microscope.

a) Disc Diffusion Method

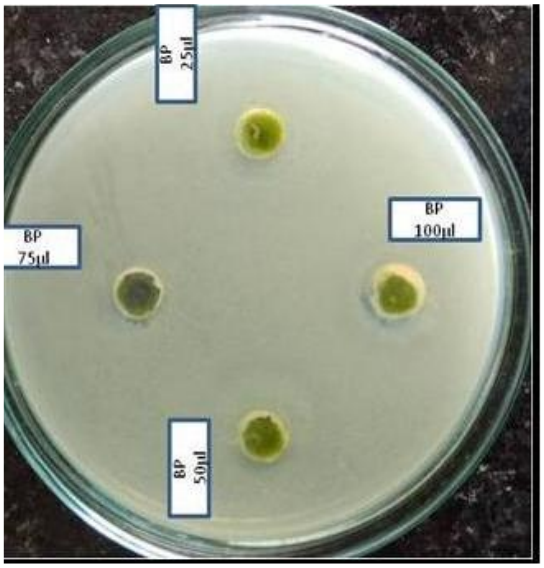
Disc diffusion method to test antibacterial activity of the extract against bacterial isolates. Broath culture of bacterial strains maintained O.D at 660nm was spread on nutrient agar media. Disc of 6mm size Whatman filter paper of different concentration of *Cyathocline lyrata* of 50, 75, 100mg/ml were placed on nutrient agar media. Disc diffused media was incubated at 37c for 8hrs. After incubation, zone of inhibition was measured using zonal scale of media

b) Well diffusion method:

100ml of conical flask of nutrient broth was inoculated with the test organism and incubated at 37C for overnight by using a sterile pipette.0.6ml of broth culture of each test organism was added to 60ml of molten agar which were cold at 45C. Mixed well and poured into a sterile petri plate.0.2ml of culture was added 20ml of agar. Agar test plate of each test organism was prepared. The agar was allowed to set and harden and required numbers of wholes were cut using a sterile cork borer ensuring proper distribution of holes in periphery and one in the culture. Agar plugs were removed. Different cork borers were used for different test organisms. Extract was used in comparison with antibacterial activity of other test organism.1mg of extract was dissolved in 1ml of triple distilled water in each hole sample was loaded. In central hole, 200µl of bacterial strain was loaded. In one hole, control was loaded and in the remaining holes, 100µl of control and 100µl of extract was loaded. Then, the plates left at room temperature for 2hrs to allow diffusion of test sample incubated face upwards at 37ºC for overnight. The diameter of zones of inhibition was measured with scale.

RESULTS AND DISCUSSION

Extract of *Cyathocline lyrata* inhibits growth of *Bacillus subtilis* at very low concentration i.e 50 µl while it inhibits growth of *Pseudomonas aeruginosa* at concentration 75µl. Oil extracted from *Cyathocline lyrata* shows better antibacterial activity at lowest concentration.



Antibacterial activity result for essential oil extract from *Cyathocline lyrata*

Bacterial	**Concentration in µl**		
	50	75	100
Bacillus subtills (NCIM 2635)	1.1mm	1.2mm	1.8mm
Pseudomonas aeruginosa (NCIM 5032)	1.3mm	-	1.5mm

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