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Research Article

Comparative pharmacognostical and phytochemical evaluation of two species of *Cyathocline*

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Abstract

In this research work, study of Comparative Pharmacognostical and Phytochemical evaluation of two species of Cyathocline were carried out. The standardization of *C. lyrata & C.purpurea* were carried out on the basis of Organoleptic, morphological characters, chemical tests, physicochemical constants, UV studies and chromatographic studies (TLC & HPLC). All there standard protocol will help in the further uses of this potent drug to incorporate in herbal formulation or used for the medication in human beings.

Keywords: *Cyathocline lyrata & Cyathocline purpurea*

1. Introduction

Plants are utilized extensively as raw drugs for many formulations in traditional systems of medicine. To check the genuineness of the raw drugs and to detect adulteration of these materials, an authentic pharmacognostic study is needed for each raw drug.

Usually the drugs are collected by traditional practitioners who have inherited Ayurvedic or other herbal practices. Their identification is mostly based on morphological features or other traditionally known characteristics. In such cases, there is a chance of selecting incorrect raw drugs/adulterants.Therefore, an extensive anatomical and phytochemical screening is needed for each raw drug used in the formulation to avoid any ambiguity and such a study will serve also as a reference for further studies.

Cyathocline lyrata & Cyathocline purpurea is well known drug in Indigeneous system of medicine for its various used as a bitter tonic. It also acts as germicide and appetizer. It is used for antibacterial, antiprotozoal, antiviral, antifungal, antifertility and pharmacological activities .*Cyathoclinelyrata*is an erect annual herb growing to 20-25 cm high branched to grooved stem has soft hair covering it. Alternately arranged stalk less leaves are toothed, covered with soft hair and 3-12 cm long flower occur in corymbs at the end of branches, flowers heads are 5-8 cm occurs and purple in colour. C*yathoclinelyrata* leaves are alternate embracing the stem, segments irregularly serrate. Heals small in panicles uniformly purple.*Cyathoclinepurpurea*is an erect annual herb, growing to 20-50 cm high. Branched, grooved stem has soft hair covering it. The whole plant is strongly aromatic. Laternately arranged stalkless leaves are toothed, covered with soft hair, and 3-12 cm long. Flowers occur in corymbs at the end of branches. Flower heads are 5-8 cm across, and purple in color. This plants are widely distributed in widespread in Himalaya (Kashmir to Bhutan), Assam, India, Burma, Thailand, Indo-China and China.The present study is based on preliminary pharmacognostic and phytochemical investigation with reference to Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) on *Cyathoclinelyrata & purpurea*.[1-5]

2. Materials and Methods

2.1 Plant material collection and authentication

The entire plant of *Cyathoclinelyrata* Cass and *Cyathoclinepurpurea* Cass was collected from local area of Betul (M.P.) in the month of June, 2012. After authentication, and voucher specimens were preserved.[5,11]

2.2 Materials and chemicals used

- **Reagents-** Molish, Fehling, Phloroglucinol, Iodine, Mayer, Dragondroff, Hager, Wagner, Biuret, sulfuric acid, hydrochloric acid, acetic acid.
- **Organic solvents** - Ethyl alcohol, methanol, hexane, ethyl acetate, chloroform, diethyl ether, toluene, petroleum ether, acetone, ammonia, n-butanol, diethyl amine, formic acid
- Precoated silica gel aluminium plate 60F-254 (20 cm X 20 cm) with 250 mm thickness.

2.3 Extraction of Plant Material[5,9]

Dried powder of plant *C. lyrata* and *C. purpurea*was successively extracted with Pet ether, chlorofrom, ethyl acetate and ethanol, filter and dried using rotary vaccumevaporater using at 40° C temp.

2.4 Determination of physiochemical Parameters

2.4.1 Pharmacognostic evaluation[15,18,22,28]

Macroscopic identification: Both thePlant material (*C. lyrata* and *C. purpurea*) has been identified for its effect on sensory organs by evaluating by necked eyes such as color, odor, taste, size, shape or any other extra feature.[9,11,21]

2.4.2 Physiochemical evaluation[14,16,26,33,34]

Phytochemical Screening: The chemical tests were performed for testing different chemical groups present in extracts.

Alkaloids: To the extract dilute hydrochloric acid was added. Then it was boiled and filtered.

i. ***Mayer's test:*** To 2-3 ml of filtrate, few drops of the Mayer's reagent was added. Formation of cream precipitate indicated the presence of alkaloids.

ii. ***Dragendorff's test:*** To 2-3 ml of filtrate, few drops of the Dragendorff's reagent was added. Formation of orange brown precipitate indicated the presence of alkaloids.

iii. ***Hager's test:*** To 2-3 ml of filtrate, few drops of Hager's reagent was added. Formation of yellow precipitate indicated the presence of alkaloids.

Flavonoids

i. Ferric-chloride test: Test solution with few drops of ferric chloride solution shows intense green colour.

ii. Alkaline reagent test: To 2 ml of test solution add 2 ml alkali, gives yellow color, which disappears on addition of dil. HCl it disappears, which indicates presence of flavonoids.

iii. Shinoda's test: In a test tube containing 0.5 ml of the extract, a small piece of magnesium was added. Then few drops of conc. hydrochloric acid was added. Formation of pink colour indicated the presence of flavonoids.

Proteins

i. Biuret's test(General test): To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

Saponins

i. Foam test: The extract was shaken vigorously with water in a test tube. Formation of persistent foam indicated the presence of saponins.

ii. Haemolytic test: Few drop of extract solution was mixed with Blood, which indicates haemolysis, shows presence of saponin.

iii. **Salkowaski test:** Concentrated sulphuric acid (2 ml) was added to 2 ml of test solution. The solution was shaken and allowed to stand. The colour of lower layer changed to yellow indicating presence of triterpenoids.

Steroids

i. Liebermann-burchard reaction: T.S 2 ml was mixed with chloroform (2 ml). To the solution, 2 ml of acetic anhydride and 2 drops of conc. Sulphuric acid from the side of test tube were added. Change in colour first red, then blue and finally green indicated presence of steroids.

Glycosides (*General test)*

Test A: 200 mg of extract were diluted with 5 ml of dilute sulphuric acid by warming on a water bath and filtered it. Then the acid extract was neutralized with 5% solution of sodium hydroxide. Then 0.1 ml of Fehling's solution A and B were added until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes. Noted the quantity of red precipitate formed and compared with that of formed in test B.

Test B: 200 mg of extract was diluted with 5 ml of water instead of sulphuric acid. Then equal amount of water (as used for sodium hydroxide in the above test) after boiling was added. Then 0.1 ml of Fehling's solution A and B were added until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes. Noted the quantity of red precipitate formed. The quantity of precipitate formed in test B was compared with that formed in test A. If the precipitate in test A was greater than in test B then glycoside may be present. Since test B represents the amount of free reducing sugar already present in the crude drug, whereas test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

Tannins

i. Ferric chloride test: Extract solutions were treated with 5% ferric chloride solution. Formation of blue colours indicated the presence of hydrolysable tannins and formation of green colour indicated the presence of condensed tannins

ii. Lead acetate test: Extract solutions were treated with 5% lead acetate solution. Formation of white precipitate indicated the presence of hydrolysable tannins

iii. Gelatin test: 3ml of test solution when treated with gelatin solution (3ml) gave white precipitate.

2.4.3 Determination of Ash Values

Determination of Total Ash: 2 g of accurately weighed root powder was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450 ºC until free from carbon, cooled and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residuewas collectedon anash less filter paper, incinerated, along with filter paper, evaporated to dryness and ignited at a temperature not exceeding 450 ºC. The ash thus obtained was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.[7,22,31,32]

Determination of Acid Insoluble Ash: The ash obtained from above procedure was boiled for 5 min. with 25 ml of dilute hydrochloric acid and the insoluble matter was collected in a Gooch crucible, or on an ashless filter paper. The insoluble matter thus obtained was washed with hot water and filter paper was ignited to a constant weight along with filter paper. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.[13,28,34]

Determination of Water Soluble Ash: The ash was boiled for 5 min. with 25 ml of water, the insoluble matter collected in a Gooch crucible, or on an ashless filter paper, washed with hot water and ignited for 15 min. at a temperature not exceeding 450 ºC. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight was the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.[11,15,22]

C. Determination of Extractive Value:

Determination of Alcohol Soluble Extractive: 5 g of the air-dried root powder was macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 hours, shaking at an interval of six hours. It was then allowed to stand for 18 hours. The macerate was filtered rapidly taking precaution against any loss of solvent. Twenty five ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105ºC to a constant weight and finally weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.[12,18,25]

Determination of Water Soluble Extractive: 5 g of the air-dried root powder was macerated with 100 ml of chloroform water of the specified strength in a closed flask for 24 hours, shaking at an interval of six hours. It was then allowed to stand for 18 hours. The macerate was filtered rapidly to prevent any loss of solvent. Twenty five ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105ºC to a constant weight and finally weighed. The

percentage of water-soluble extractive was calculated with reference to the air-dried drug.[17,18,27]

D. Determination of Moisture Content (loss on drying): About 10 g of root (without preliminary drying and cut in parts of about 3 mm in thickness), after accurately weighing (weight to within 0.01g) was placed in a tarred evaporation dish. It was then dried at 105ºC for 5 hours and weighed. Drying was continued and the root was weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30 min. and cooling for 30 min. in a desiccator, did not show more than 0.01g difference.[6,23,27,30]

2.4.4 Determination of Analytical Parameters Through following instrument

2.4.4.1 Thin Layer Chromatography: Thin layer chromatography: T.L.C. is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase.[13,17,26]

2.4.4.2 U.V. Spectrophotometry: Scraped sample of T.LC was dissolved in methanol and determined the λmax of Flavonoids as compared to sample spot found in thin layer chromatography.[7,11,19]

2.4.4.3 High Performance Liquid Chromatography: Initially to estimate Quarcetin number of mobile phase in different ratio were tried.

The mobile phase found to be most suitable for analysis was 50Mm KH_2PO_4 Buffer (pH-3 with OPA): Acetonitrile in the ratio of (30**:**70 v/v). The mobile phase was filtered through 0.45m filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min and wavelength 360 nm.[23,29,33]

3. Result and Discussion

Preliminary phytochemical screening of ethanolic and aqueous extracts of *Cyathocline lyrata* revealed the presence of alkaloids, tannins and flavonoids while ethanolic and aqueous extract of *Cyathocline purpurea* revealed the presence of flavonoids and glycosides.

Table 1. Phytochemical screening of extracts *Cyathocline lyrata*

Chemical Tests	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Alkaloids				
Mayer's reagent	-	-	-	+
Hager's reagent	-	-	-	-
Wagner's reagent	-	-	-	-
Dragendorff's reagent	-	-	-	+
Glycosides (+Ve)				
Baljet test	-	-	-	+
Legal's test	-	-	-	+
Keller-Kiliani	-	-	-	+
Phenols/Tannins				
Ferric chloride	-	-	+	+
Gelatin Solution	-	-	+	+
Lead acetate test	-	-	+	+
Flavonoids				
$FeCl_3$ test	-	-	+	+
Alkaline reagent test	-	-	+	+
Shinoda test	-	-	+	+
Saponins				
Foam test	-	+	-	+

Hemolytic test	-	+	-	+
Lead acetate	-	+	-	+
Fixed oil/Fats				
Spot	+	-	-	-
Saponification	+	-	-	-
Gums & Mucilage				
Water	-	-	-	-
Carbohydrates				
Molish test	-	-	-	-
Fehling's solution test	-	-	-	-
Benedict's test	-	-	-	-
Amino acids				
Ninhydrin Test	-	-	-	-
Millons Test	-	-	-	-
Xantoprotein Test	-	-	-	-
Terpenoids				
Lieberman Burchard Test	+	+	-	-
Salkowski test	+	+	-	-
Steroids				
Lieberman Test	-	+	-	-
Protein				
Biuret test	-	-	+	+

Table 2. Phytochemical screening of extracts *Cyathocline Purpurea*

Chemical Tests	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Alkaloids				
Mayer's reagent	-	-	-	+
Hager's reagent	-	-	-	+
Wagner's reagent	-	-	-	-
Dragendorff's reagent	-	-	-	+
Glycosides (+Ve)				
Baljet test	-	-	-	+
Legal's test	-	-	-	+
Keller-Kiliani	-	-	-	+
Phenols/Tannins				
Ferric chloride	-	-	+	+
Gelatin Solution	-	-	+	+
Lead acetate test	-	-	+	+
Flavonoids				
$FeCl_3$ test	-	-	+	+
Alkaline reagent test	-	-	+	+
Shinoda test	-	-	+	+
Saponins				
Foam test	-	+	-	+

Hemolytic test	-	+	-	+
Lead acetate	-	+	-	+
Fixed oil/Fats				
Spot	+	-	-	-
Saponification	+	-	-	-
Gums & Mucilage				
Water	-	-	-	-
Carbohydrates				
Molish test	-	-	-	-
Fehling's solution test	-	-	-	-
Benedict's test	-	-	-	-
Amino acids				
Ninhydrin Test	-	-	-	-
Millons Test	-	-	-	-
Xantoprotein Test	-	-	-	-
Terpenoids				
Lieberman Burchard Test	+	+	-	-
Salkowski test	+	+	-	-
Steroids				
Lieberman Test	+	+	-	+
Protein				
Biuret test	-	-	+	+

Table 3. Comparative Phytochemical parameter of *cyathocline lyrata*&*cyathocline purpurea*

S. No.	Parameter		Results	
			Cyathocline lyrata	***Cyathocline purpurea***
1	Extraction	Pet. ether	1.80%	1.85%
		Chloroform	1.23%	0.85%
		Ethyl acetate	2.03%	2.85%
		Ethanol	4.43%	5.26%
2	Pharmacognostic evaluation	Colour	Dark Brown color	Brown color
		Odour	Aromatic characteristic	Aromatic characteristic
		Taste	Bitter	Bitter
		Texture	Fine and fibrous	Fine and fibrous
3	Ash value	Total ash	4.07%	3.8%
		Acid Insoluble	1.60%	1.20%
		Water soluble	03.48%	2.56%
4	Extractive Value	Alcohol soluble	13.20%	12.5%
		Water soluble	08.63%	7.73%
		Ether soluble	03.48%	2.56%
5	Moisture Content		2.87%	3.56 %
6	RfValue		0.43	0.42
7	% Assay	HPLC	0.11	0.07

Fig 1. HPLC Graph of *Cyathocline Purpurea*

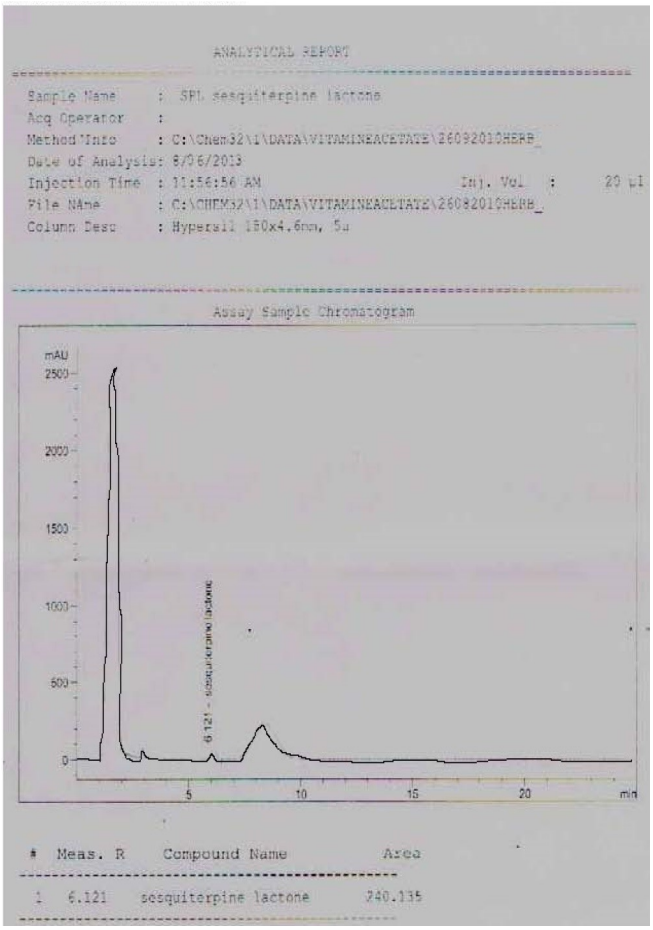
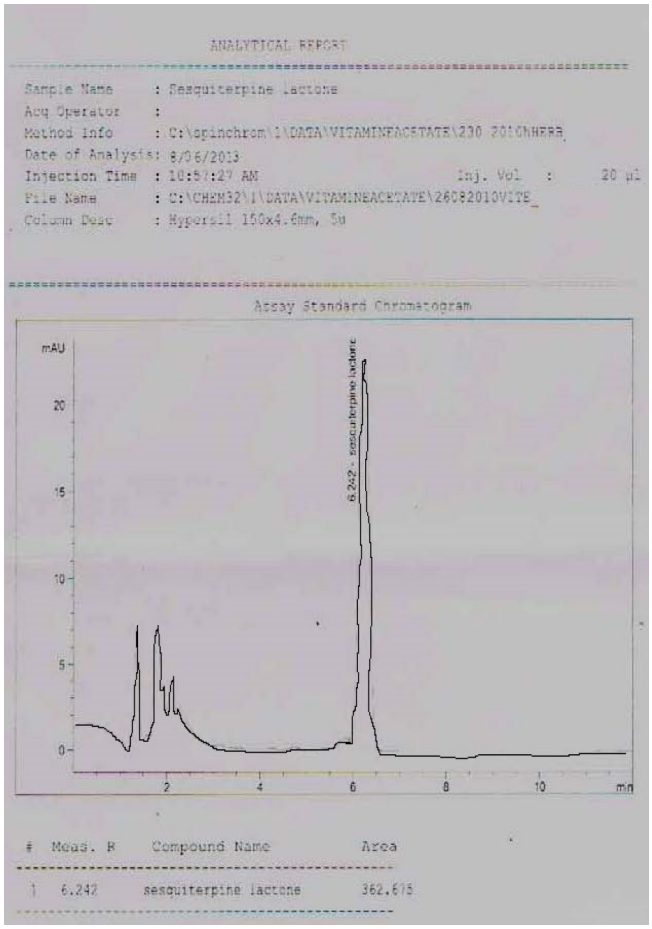


Fig 2. HPLC Graph of *Cyathocline Lyrata*



4. Conclusion

To meet this new thrust of inquisitiveness, standardization of Indian system of medicine is mandatory, for that the Herbo-mineral formulations and their Quality control development was carried out. The formulation contains different type of ingredients in the form of herbs and minerals. The individual drugs were standardized pharmacognostically, physicochemical and analytically (TLC, HPLC & UV). From the studies, it was found that all the ingredients meet there specifications.

The standardization of *C. lyrata*& *C. purpurea* were carried out on the basis of Organoleptic, morphological, microscopical characters, chemical tests, physicochemical constants, UV studies and chromatographic studies (TLC & HPLC). Thus from the studies done so far, it may be concluded that the general protocol for Standardization of *C. lyrata*& *C. purpurea* which includes macroscopic characters, Physiological characters: Identification, Ash value, Extractive value, Moisture content, Assay value by HPLC etc., Phytochemical and analytical Parameters. All there stander protocol will help in the futher uses of this potent drug to incorporate in herbal formulation or used for the medication of human beings.

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