THERAPEUTIC USES OF CYANTHILLIUM CINEREUM

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Abstract
The whole plant of *Cyanthillium cinereum* has several pharmacological properties in treating a broad range of diseases in traditional medicine but underestimated to be used as a commercial drug. As a remedy to several health conditions, phytocompounds of *C. cinereum* can be used as multi-target drugs to treat comorbidity. The present study deals with the phytochemical, HPLC and antimicrobial role of the methanol leaf extract of this plant. It was observed that there was very good presence of alkaloids, saponins, fixed oils and moderate presence of terpenoids, carbohydrates and polyphenols which are known for their medicinal roles. The antimicrobial roles of the methanol extract were poor compared to the respective standards used. It is concluded that the medicinal properties of this plant could be due to the presence of various phytocostituents.

Keywords: *Cyanthillium cinereum,* Phyto-compounds, multi-target drugs, traditional medicine, alternative *medicine,* Saponns

Introduction

*Cyanthellium cinereum*commonly called the little ironweed, belongs to the family of Asteraceae and has become a native to Africa, Australia, tropical and temperate Asia though originally a native of Central America. *C. cinereum* commonly occurs in upland crop areas, gardens, waste places, and along the roadsides and its therapeutic benefits are often underrated. Studies on phytochemical constituents of *C. cinereum* have shown the presence of glycosides, flavonoids, aliphatic acids,terpenoids, sterols, saponins, tannins, fatty oils, triterpenoids, alkaloids, esters, and sesquiterpenes on extraction with different solvents [1]. The major phytoconstituents present in the ethanoic extract of *C. cinereum* are lupeol, lupeol acetate, luteolin-7-O-glucoside, stigmasterol-β-D-glucopyranoside, stigmasterol and dotriacontanoic acid, along with several other minor phytochemicals [2][3].

The extensive free radical scavenging potential of dried whole plant *C. cinereum* is well studied and established that might contribute to its total phenolic and flavonoid contents resulting in a therapeutic benefit [4][5]. Extract of *C. cinereum* has been used in the treatment of urinary incontinence and piles. Also, it is given as a decoction to treat diarrhea, stomachache, cough, and bronchitis in traditional medicine, without any adverse effects. [6]. *C. cinereum* possesses anti-microbial, anti-bacterial properties and has been used as an alternative source to antibacterial agents [7][8].The whole plant has several pharmacological properties in treating a broad range of diseases in traditional medicine but underestimated to be used as a commercial drug. This review elaborates on the therapeutic benefits of the herb, *C. cinereum* and its prospects in treatment of diseases.

PHYTOCHEMICAL ANALYSIS: QUALITATIVE

The following tests were performed on the methanol extract of *Cyanthilliumcinereum* leaves to detect various phyto-constituents present in them (Trease and Evans, 1983)

Alkaloids - Dragendorff's test

Solvent free extract (50 mg) was stirred with few mL of dilute hydrochloric acid and filtered.
To a few mL of filtrate, 1or 2 mL of Dragendorff's reagent was added. A prominent yellow precipitate indicate the test as positive.

Carbohydrates - Fehling's test

The extract (100 mg) was dissolved in 5 mL of water and filtered. One mL of filtrate was boiled on water bath with 1 mL each of Fehling solutions I and II. A red precipitate indicated the presence of sugar.

Glycosides - Borntrager's test

The extract (50 mg) was hydrolysed with concentrated HCl for 2 h on a water bath, filtered and to the 2 mL of filtrate hydrolysate 3 mL of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

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Saponins - Foam test
The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken for 15 min. A two cm layer of foam indicated the presence of saponins.

Proteins - Biuret test
An aliquot of 2 mL of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

Aminoacids - Ninhydrin test
Two drops of ninhydrin solution were added to two mL of aqueous filtrate. A characteristic purple color indicated the presence of amino acids.

Phenolic compounds – Ferric Chloride test
The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Fixed oils
A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

Terpenoids
To 1 mL of extract, 2 mL of trichloroacetic acid (TCA) was added and the formation of yellow to red precipitate showed the presence of terpenoids.

PHYTOCHEMICAL ANALYSIS: QUANTITATIVE

Determination of total phenolic content
The method ofSingleton and Rossi, 1965 was employed for this estimation. The plant powder (2 g) was soaked in methanol and kept in the orbital shaker for 24 hrs. The residues were then filtered and the filterate was evaporated. The extract was centrifuged at 10,000 rpm for 15 min at 4°C. 20 µL of the extract was made up to 3 mL using distilled water. 0.5 mL of Folin- Ciocalteu's phenol reagent was added to the tubes and placed in the incubator for 3 min at 45°C. After 3 min, 2 mL of 20% Na_2CO_3 was added to the tubes and kept for incubation after which, absorbance was measured at 650 nm. The total phenol content in the sample was calculated using the formula,

$$\mathbf{C\ (GAE) = c\ x\ V/M}$$

where, c = concentration of sample from the curve obtained (mg/mL), V = volume used during the assay (mL) and M = mass of the sample used during the assay (g).

Determination of total flavonoids
Flavonoid contents were determined by slightly modified spectrophotometry method of Karadeniz *et al*. (2005). One g of dry powder was weighed and ground with 200 mL of 80 % aqueous methanol in a mortar and pestle. The ground sample was filtered and a clear filterate was obtained. The aliquot of the sample (0.5 mL) was taken in a test tube, add 3 mL of distilled water and 0.3 mL of 5% sodium nitrite were added. The solution was vortexed and allowed to stand at room temperature for 5 min. and 0.6 mL of 10% aluminium chloride was added to the solution. After
6 min, 2 mL of 1 M sodium hydroxide was added to the test tube. The solution was made up to 10 mL with distilled water. The absorbance was read at 510 nm. The total flavonoid content was calculated as quercetin equivalent (mg QE/g) using the formula,

$$\mathbf{X = (A.M_0/A_0.M)}$$

where, A= absorption of sample, A_0= absorption of standard (quercetin), M= weight of sample (mg/mL) and M_0= weight of quercetin in solution (mg/mL)

HPLC ANALYSIS
HPLC analysis of methanolic extract of the samples was carried out with Chromatographic system (YL 9013, Japan) consist of an autosampler (YL 9528) with 100µl fixed loop and an YL 9163 UV-Visible detector. The separation was performed on a CTO-10A column at an ambient temperature, a CBM-10A interface and a LC-10 Workstation. The mobile phase consists of methanol: water (70:30 v/v) and the separations were performed by using isocratic mode, elution performed at a flow rate of 1 mL/min. The samples were run for about 20 min each and the detection was done at 274 nm by UV detector. All the chromatographic data were recorded and processed using autochro-3000 software.

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ANTIMICROBIAL ACTIVITY

Antimicrobial assay of different samples was performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 × 108 CFU/ml. MHA plates was cultured with standardized microbial culture broth. Each well was filled with varying concentrations from 150-200 µg/ml of the samples with positive control as streptomycin 25 mcg and negative/solvent control as 100% DMSO, respectively. The plate was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of the tested samples. The zone of inhibition (ZOI) was observed and measured in mm. (Wyne, 2015)

RESULTS AND DISCUSSION

The results of qualitative analysis of methanol extracts of *Cyanthillium cinereum*leaves are depicted in Table1. It was found that except for terpenoids and glycoside, other phyto-constituents were present. Alkaloids, Saponins, fixed oils were present in more concentration whereas terpenoids, carbohydates and phenolic compounds were present in smaller concentrations. There was a total lack of amino acids, proteins and glycosides. The presence of these phytoconstituents could be instrumental in the medicinal effects of this plant.

Table 1. Indicates the presence or absence of phyto-constituents in the methanol extract of *Cyanthillium cinereum*leaf extract.

Phytocomponent	Degree of Presence
Alkaloids	+++
Terpenoids	+
Saponins	+++
Fixed Oils	+++
Glycosides	--
Carbohydrates	++
Phenolic Compounds	++
Proteins	--
Aminoacids	--

+: Phytocomponents present at lower concentration
++: Phytocomponents present at medium concentration

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+++: Phytocomponents present at higher concentration
---: Phytocomponents absent

The methanol extract of *Cyanthillium cinereum* leaves indicated the 44.5 mg/g and 129 mg/g of phenols and flavonoids, respectively as shown in Table 2.
Table 2. Indicates the Quantitative Analysis results of methanol extract of leaves of *Cyanthillium cinereum*

Phytochemical	Results
Total Phenol (mg/g)	44.5
Total Flavonoid (mg/g)	129

ANTIMICROBIAL ACTIVITY
The antimicrobial activity of the methanol extract of *Cyanthilliumcinereum* leaves is shown in Table 3 and Figure 2. From the results it is observed that at a concentration of 100 µg, the plant sample did not show any antimicrobial activity. At 150 and 200 µg concentration there was some activity but as compared to both streptomycin (for bacteria) and Kanamycin (for fungus) the activity was less. By increasing the concentrations we expect to get better antimicrobial action of this plant extract.
Table 3 Indicates the Zones of Inhibition for each concentration of the sample of *Cyanthillium cinereum* methanol leaf extract and respective antibiotics against the microorganisms.

Table 3. Indicates the Zones of Inhibition for each concentration of the sample and respective antibiotics against the microorganisms.

Organisms	**100 µg**	**150 µg**	**200 µg**	**Streptomycin 25 µg**
E. coli	**--**	**4 mm**	**8 mm**	**11 mm**
PA	**--**	**3 mm**	**5 mm**	**11 mm**
SA	**--**	**--**	**4 mm**	**11 mm**
CA	**--**	**--**	**3 mm**	**Kanamycin 25 µg 11 mm**

Bacteria: E. coli: *Escherichia coli*; **PA:** *Pseudomonas aeruginosa*; **SA:***Staphylococcus aureus.* **Fungus: CA:***Candida albicans*

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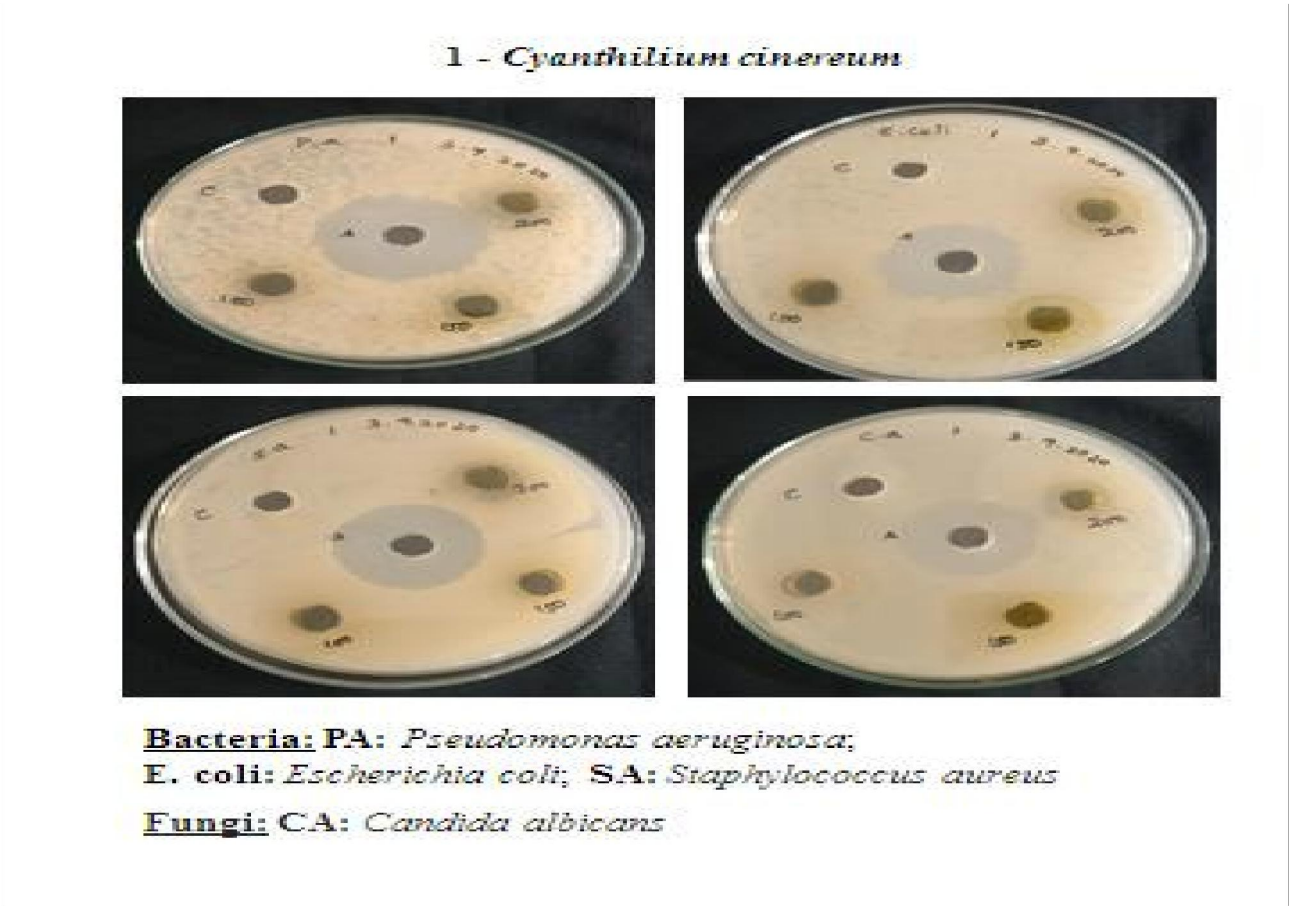
Figure 2. Indicates the antimicrobial role of methanol extract s of the leaves of *Cyanthillium cinereum*



HPLC Analysis

The HPLC analysis of the methanol extract of the sample was limited to 5 µg L-1 and a flow rate of 1 mL/min showed good detection of component present with low noise level with a retention time of 3. 893 in a time interval less than 5 minutes Figure 1.

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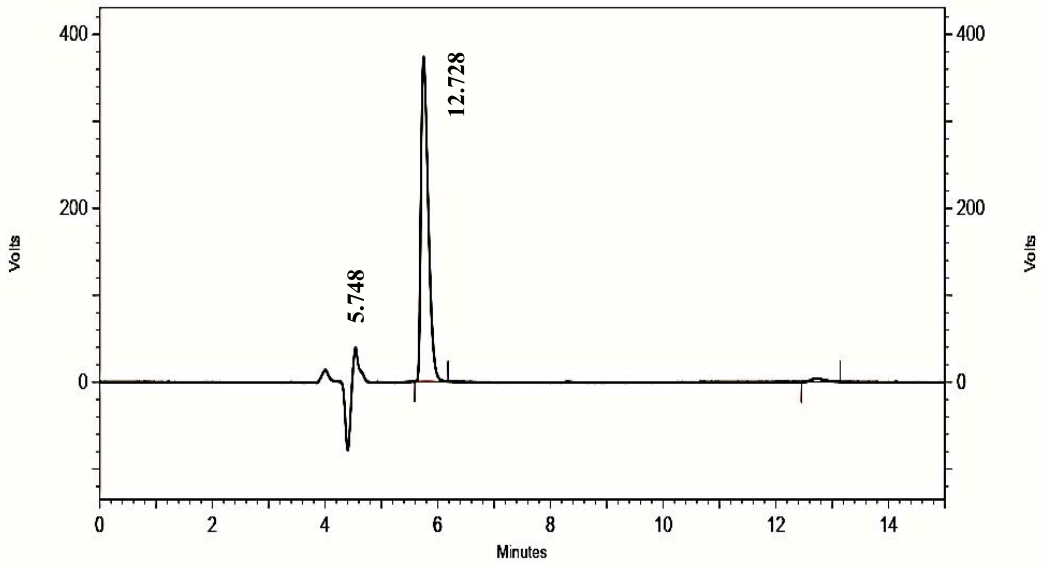
Figure 1 represents the HPLC profile of methanol leaf extract of *Cyanthillium cinereum*
Sample: *Cyanthilium cinereum*

Method: C:\ChromQuest\Enterprise\Projects\Default\Method\Project.met



Name	Retention Time	Area	Area %
	5.748	3339770	98.09
	12.728	64967	1.91

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