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ASSESSMENT OF PHYTOCHEMICALS, ANTIOXIDANT AND ANTIMICROBIAL EFFICACY FROM DIFFERENT SOLVENT EXTRACTS OF A MEDICINAL PLANT - OF *Cyanthillium cinerium* (L.) H. Rob.

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Abstract: In this study, the antioxidant and antimicrobial potential of *Cyanthillium cinerium* (L.) H. Rob. whole plant crude extract was investigated. Polar solvents (methanol, aqueous) and non-polar solvent (hexane) was used, to extract the biologically active components from the plant sample by adopting hot continuous soxhlet extraction method. Phytocompounds such as alkaloids, carbohydrates, saponins and phenolic compounds were chiefly identified in the methanol and aqueous extracts. The hexane extract showed only the presence of phenolic compounds. The quantity of total phenolic content was found to be high in methanol extract (4.45 mg/g), followed by aqueous extract (3.2 mg/g) and hexane extract (1.4 mg/g). Similarly, total flavonoids were high in methanol extract (1.20 mg/g). The quantity of flavonoid was (0.20 mg/g) and (0.10 mg/g) in the hexane and aqueous extracts respectively. Antioxidant radical scavenging activity conducted with DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and phosphomolybdenum assay for the crude solvent extracts showed potent results. The DPPH % inhibition expressed by methanol extract was IC50 27.23 µg/mL and ABTS % inhibition was found to be IC50 58.98 µg/mL. The free radical scavenging potential of methanol extract with the phosphomolybdenum assay showed highest absorbance of 1.211nm at 100 µg/mL. Aqueous extract possessed high ion chelating activity, IC50 value of 46.87 µg/mL. The crude extract of the plant was screened for its antimicrobial zone of inhibition against five human pathogenic organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, Methicillin resistant *Staphylococcus aureus* and *Candida albicans*. Maximum zone of antimicrobial inhibition of 5 mm and 3 mm was exhibited by 150 µg/mL of methanol extract followed by hexane extract against *Pseudomonas aeruginosa* and Methicillin resistant *Staphylococcus aureus*, respectively.

Key Words **- DPPH, ABTS, Zone of Inhibition, Free Radical Scavenging, Flavonoids, Phenols.**

I. INTRODUCTION

Cyanthillium cinereum (L.) H. Rob. is a weed crop and commonly referred as little ironweed. It belongs to the Angiosperm family Compositae (Asteraceae). The plant is widely distributed across the tropical and subtropical regions of the world. Leaves are rich in medicinal properties and used as analgesic, anti-pyretic, anti-bacterial and anti-fungal agents (Yusoff *et al*., 2020). Due to its enormous medicinal value it is traditionally used in *Ayurveda* to treat fever. The plant decoction or infusion provides remedy for spasms of the urinary bladder and strangury. It also has therapeutic potentials against asthma, cancer, cholera, colic pain, cough, diarrhea, dysentery, impotency and night-blindness (Joshi *et al.* 2021). Iwalewa *et al*. (2003) reported that *Cyanthillium cinereum* (L.) H. Rob. plant contains antioxidant compounds like tannins, catechins, and flavonoids which shields 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) oxidation in human red blood cells.

Phytocompounds such as phenolic, vitamin, amino acids, minerals etc., can be used as potential therapeutics for cancer, cardiovascular and other chronic diseases. Polar solvents (water, ethanol and methanol) are commonly used for phenolic compounds extraction, while non-polar solvents (hexane, chloroform, petroleum ether) are used for oil and fat extractions (Ketsuwan *et al.* 2017). Water and ethanol contain hydroxyl group and can form hydrogen bonding with the bioactive compounds. Aqueous extraction is found more effective than ethanol extraction in antimicrobial compound because water has higher polarity and shorter chain than ethanol (Pin *et al*. 2010).

Naturally occurring antioxidants are used in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity. Antioxidants prevent certain types of chemical damages caused in cells by an excess of free radicals, charged molecules produced due to smoking, exposure to pesticide and fumes from exhaust. Free radicals annihilation gives protection against cancer, heart diseases, stroke and other immune compromising diseases (Yi-Fang *et al*. 2002; Arouma, 2003).

According to the reports of World Health Organization (WHO), 70% of the world's population rely on medicinal plants for primary health care, which are biologically active with desirable antimicrobial and antioxidant properties (WHO, 2008). The medicinal value of phytoconstituents is because of the presence of chemical substance that produces definite physiological action on the human body (Mahesh and Satish, 2008; Hamid *et al*. 2010). Compounds like alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium enhance cell growth, replacement and body building (Bouamama *et al*. 2006). Since two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search for new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures (Okemo *et al*. 2003; *Arora* and Kaur, 2007).

Hence, the aim of the present work was to analyse the phytocompound characters of *Cyanthillium cinereum* (L.) H. Rob. plant extracts obtained using polar and non polar solvents, and evaluate its chemical constituents for antioxidant and antimicrobial efficiencies.

II. MATERIALS AND METHODS

2.1. Plant material

Cyanthillium cinereum (L.) H. Rob plant was collected from Thiruvallur district, Tamil Nadu, India, in the month of February, 2021. The plant sample was identified and authenticated by Dr. Abdul Kadher, Associate Professor, Department of Botany, Presidency College, Chennai, Tamil Nadu, India (Fig.1. a & b). Fresh, healthy plant samples were washed twice under running tap water and then rinsed with distilled water, to remove the external debris adhered on its surface. The washed plant material was shade dried at room temperature to get rid of residual moisture. The dried plant sample was cut into smaller pieces and pulverised into a fine powder. The powdered plant sample was sieved and was stored in an air tight sterile container and later used for the preparation of plant extracts.

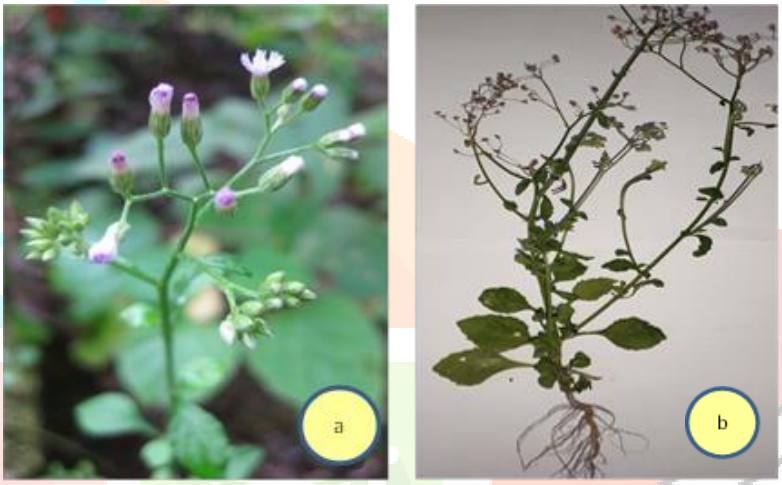

Fig.1.a. habitat of *Cyanthillium cinerium*
1.b. whole plant of *Cyanthillium cinerium*

2.2. Preparation of plant extracts

Biologically active components from the powdered plant sample were extracted using polar and non-polar solvents such as methanol, aqueous and hexane. This process was carried out under hot continuous extraction technique using soxhlet apparatus. Accurately weighed 30g of plant powder was added in 3L of methanol, 10g of plant powder was added in 2L of aqueous solvent and 7g of plant powder was added in 3L of hexane. Each consequent phytoextract was evaporated to dryness at 60°C – 90°C using rotary evaporator, until the solvent layer was completely volatilized. The concentrated extract was further used for screening the phytochemicals, antioxidant and antimicrobial activities.

2.3. Qualitative phytochemical analysis

The crude plant residues, extracted using methanol, aqueous and hexane solvents were screened qualitatively for the presence of various classes of biologically active chemical components such as, saponins, flavonoids, terpenoids, glycosides, alkaloids, proteins, amino acids, carbohydrates and phenols adopting the following protocols (Trease and Evans, 1989).

2.3.1. *Test for Saponins:* 5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The foam appearance showed the presence of saponins.

2.3.2. *Tests for Flavonoids*: *Alkaline Reagent Test*: 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced. This result showed the presence of flavonoids.

2.3.3. *Test for Terpenoids*: 2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A grey color formed which showed the entity of terpenoids.

2.3.4. *Tests for Glycosides:* A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 ml aqueous plant extract and 1 ml H_2SO_4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

2.3.5. *Test for alkaloids:* Dragendorff's test, 5 mg extract was taken in tube. Add one drop of dragendorff's reagent and orange-red color precipitate shows the presence of alkaloids.

2.3.6. *Test for proteins:* Biuret's test- 5 mg extract was added with the few drops of biuret's reagent. The obtained mixture was shaken well and allowed to warm for 1-5 min. Appearance of red or violet colour indicated presence of proteins.

2.3.7. *Test for Amino acids:* Ninhydrin test- 5 mg extract of the sample was mixed with 2 ml of 0.2% solution of Ninhydrin and boiled for 2 min on water bath, if violet colour appeared with the presence of amino acids.

2.3.8. *Test for carbohydrates*: Fehling's test-5 mg extract was mixed with few drops of benedict's reagent, than allowed to boiled, the reddish brown precipitate are found with the presence of the carbohydrates.

2.3.9. Test for phenols: To 20 ml of distilled water in a test tube, the powdered sample of leaves is boiled and then filtered. Add 3-4 drops of 0.1% v/v Ferric chloride to the filtered sample and the color changes to brownish green or blue, it indicates presences of phenols.

2.4. Quantitative phytochemical analysis

The amount of phenols and flavonoids present in the plant extracts was quantitatively analyzed.

2.4.1. *Determination of total phenolic content* (Singleton and Rossi, 1965)

The plant powder (2 g) was soaked in different solvents such as methanol, aqueous and hexane were kept in the orbital shaker for 24 hrs. The residues were then filtered and the filterate was evaporated. The different extracts of plant material were then centrifuged at 10,000 rpm for 15 min at 4°C. Twenty µL of extracts was prepared using the supernatant and made up to 3 mL of distilled water. Then, 0.5 mL of Folin- Ciocalteu's phenol reagent was added to all the tubes. The tubes were then placed in the incubator for 3 min at 45°C. After 3 min, 2 mL of 20% Na_2CO_3 was added to all the tubes and kept for incubation after which, its absorbance was measured at 650 nm. The total phenol content in the sample was calculated using the formula,

$$C\ (GAE) = c \times V/M$$

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)

2.4.2. *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,

$$X = [A.M_0/A_0.M]$$

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)

2.5. Antioxidant assay

2.5.1. *DPPH Assay*

The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Szabo *et al*. (2007). The samples were reacted with the stable DPPH radical in methanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 1 mL of methanol and 1 mL of DPPH radical solution 0.5mM in methanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 30 min of reaction using a UV-VIS spectrophotometer. The control solution was prepared by mixing methanol (1.0 mL) and DPPH radical solution (1.0 mL) and 1 mL of methanol serves as blank. The scavenging activity percentage was determined according to

$$\%\ \text{of inhibition} = [\text{Control O.D} - \text{Sample O.D}] / \text{Control O.D} \times 100.$$

2.5.2. *ABTS Radical Scavenging Assay*

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolourization assay. ABTS+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS·+ solution was then diluted with methanol and the absorbance was read at 734 nm. After the addition of 5 µl of plant extract to 3.995 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula, ABTS·+ scavenging effect (%) = {[AB–AA]/ AB}×100 ,where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard. Ascorbic acid was used as standard substance (Pellegrini *et al*. 1999).

2.5.3. *Phosphomolybdenum Assay*

Extracts in different concentration ranging from 10 to 100 µg/mL were added to each test tube individually containing 1 ml of distilled water and 1 ml of Molybdate reagent solution, 1mL of Sodium phosphate and 1 mL of Sulphuric acid were added separately. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. The values were recorded (Prieto *et al*. 1999).

2.5.4. *Ion Chelating Assay*

The chelation of ferrous ions by extracts was estimated by method of Dinis *et al*. (1994). Briefly, 50 µl of 2 mM FeCl2 was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as [(A0- As)/ As] × 100, where A0 was the absorbance of the control, and as was the absorbance of the extract/ standard. Na_2EDTA was used as positive control (Dinis *et al*. 1994).

2.6. Antimicrobial Assay

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 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 (Daoud *et al*. 2015).

III. RESULTS AND DISCUSSION

3.1. Phytochemical extraction using organic solvents

Preliminary phytochemical screening of *Cyanthillium cinereum* (L.) H. Rob., plant crude extract obtained by different solvents systems produced variations in the percentage of phytocompound yield (Table 1). The methanol solvent extract revealed the presence of alkaloid, carbohydrates, saponin and phenolic compounds. It was devoid of glycosides, protein and amino acids. The hexane solvent extract showed only the presence of phenolic compounds and the other phytocompounds such as alkaloids, carbohydrates, glycosides, saponin, protein and amino acids were absent in the hexane extract. The aqueous extract of the powdered plant sample showed the presence of alkaloids, carbohydrates, saponin and phenolic compounds and was devoid of glycosides, proteins and amino acids. From the qualitative tests, it could be seen that phenolic compounds were present in the extracts for all the 3 type of solvents used. Glycosides, proteins and amino acids were absent in all the three solvent extracts. Alkaloids, carbohydrates and saponin were present only in methanol and aqueous extract and were absent in hexane extract. Preliminary phytochemical evaluation of medicinal plants, help in the study of novel drugs and isolation of its pharmacological active principles. All the phytoconstituents extracted from plant possessed potent medicinal properties, associated with self-dense mechanism against microbes and pathogens. It is evident from its antioxidant and antimicrobial type biological activities. The percentage yield of crude extract and bioactive compounds from the plant sample varied due to different types of solvents used in the extraction process. Also the polarity of the solvent had significant impact on the solubility of the extracted bio compounds (Naima *et al*. 2015). The chemical structure of bioactive compound also showed impact on its extraction depending on the dielectric constant of solvent used, such as for methanol 32.70, water 80.4 and hexane 1.88 (Felhi *et al*. 2016a). Singh *et* al. (2014) in a study reported the presence of carbohydrate, tannin, saponin, terpenoids phytoconstituents in *Vernonia cinerea* leaves extracted using various polar and non-polar solvent systems.

Table 1. Preliminary phytochemical screening of *Cyanthillium cinerium* (L.) H. Rob.

TESTS	Methanol extract	Hexane extract	Aqueous extract
Alkaloids – Dragendorff's Test	+++	---	+++
Carbohydrates – Fehling's Test	+++	---	+++
Glycosides – Borntrager's Test	---	---	---
Saponin – Foam Test	+++	---	+++
Protein – Biuret Test	---	---	---
Amino acid – Ninhydrin Test	---	---	---
Phenolic compounds – Ferric Chloride Test	+++	+++	+++

Note - (+++) present (---) absent

3.2. Quantitative Phytochemical Analysis

3.2.1. *Total Phenolic content determination*

The results of total phenolic content in the methanol extract, aqueous extract and hexane extract at 650 nm absorbance was determined (Table 2). The highest concentration of phenolic compound 4.45 mg/g was present in methanol extract, followed by aqueous extract 3.2 mg/g. The hexane solvent produced the least amount, 1.4 mg/g of phenolic content from the plant material. Plant phenolic compounds are very significant, because these phytocompounds groups contain free radicals scavenging abilities. Sankhalkar and Vernekar, (2016) in a study reported 2.28 mg/g and 2.18 mg/g of total phenolic compound in the methanol extract of *Moringa oleifera* Lam and *Ocimum tenuiflorum* L. leaves.

3.2.2. *Total Flavonoid content determination*

The results for total flavonoids content (Table 2) at 510 nm absorbance was 1.20 mg/g concentration with methanol extract, followed by 0.20 mg/g with hexane extract and 0.10 mg/g with aqueous extract. The flavonoid content of methanol extract of *Moringa oleifera* Lam and *Ocimum tenuiflorum* L. leaves were reported to be 4.45 mg/g and 4.47 mg/g respectively by Sankhalkar and Vernekar, (2016).

Table 2. Quantitative Phytochemical Analysis of *Cyanthillium cinerium (L.) H. Rob.*

Quantitative analysis	Methanol extract	Hexane extract	Aqueous extract
Total Phenolic Content Determination			
Absorbance at 650nm	0.852	0.267	0.611
Concentration (mg/g)	4.45	1.4	3.2
Total Flavonoids Content Determination			
Absorbance at 510nm	0.908	0.155	0.103
Concentration (mg/g)	1.20	0.20	0.10

3.3. Antioxidant Activity

3.3.1. DPPH Assay

DPPH reduction, by free radical scavenging potential of methanol, hexane and aqueous extracts of *Cyanthillium cinereum* (L.) H. Rob. was analyzed (Table 3). All the extracts produced considerable percent of DPPH free radical scavenging activity and are represented by IC_{50} values. Among the three solvent extracts, methanol extracts showed highest scavenging potential with IC_{50} value of 27.23 µg/mL, while standard quercetin at the same concentration produced 6.14% of inhibition. The hexane extract showed IC_{50} value of 28.86 µg/mL reductions and aqueous extract showed IC_{50} value of 78.74 µg/mL reducing activity (Fig.2a, 2b). The aqueous extract showed poor free radical scavenging activity. From the results it is evident that the plant components had good antioxidant potential when compared with standard quercetin. The effect of antioxidants of the plant extracts on DPPH is thought to be due to the hydrogen donating ability of plant chemical constituents. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis (Huang *et al.* 2005). The results of the study showed that the different solvent extracts of the plant leaves produced radical scavenging ability either by electron transfer or ability to donate hydrogen molecule. Rahman *et al.* (2015) reported similar type of free radical scavenging ability in DPPH assay by the methanol extract of *Tabebuia pallida* whole plant.

Table 3. Antioxidant activity by DPPH Assay

Conc. (µg/mL)	% of Inhibition		
	Methanol extract	Hexane extract	Aqueous extract
10	21.81	40.74	19.49
20	28.65	47.66	26.29
30	55.08	51.96	28.44
40	55.63	61.12	29.51
50	75.41	61.86	34.05
60	77.07	63.92	39.17
70	78.92	65.04	47.22
80	81.33	68.03	50.80
90	82.99	75.14	60.10
100	84.28	76.82	66.90
IC_{50} value	**27.23**	**28.86**	**78.74**

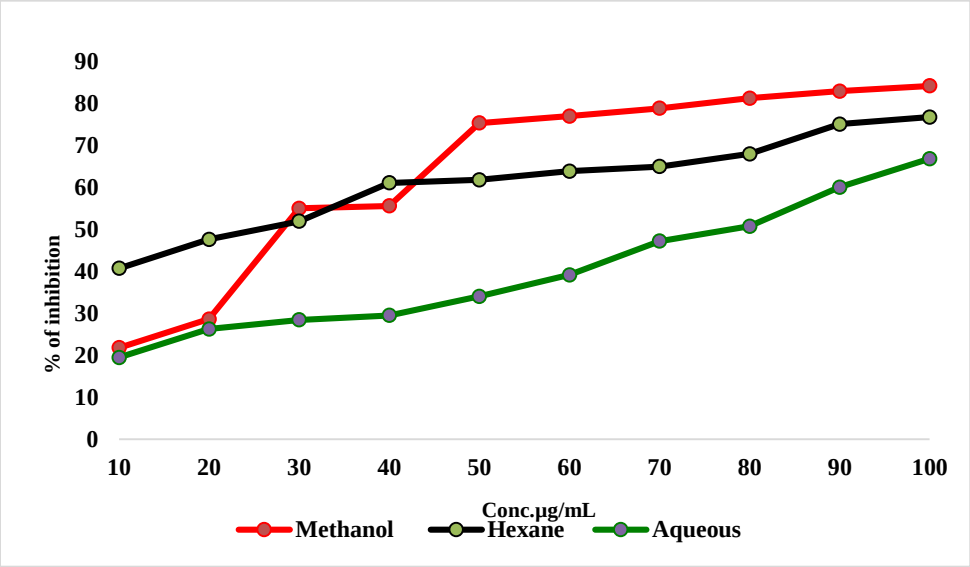

Fig.2a. DPPH activity of extracts of *C. cinerium*

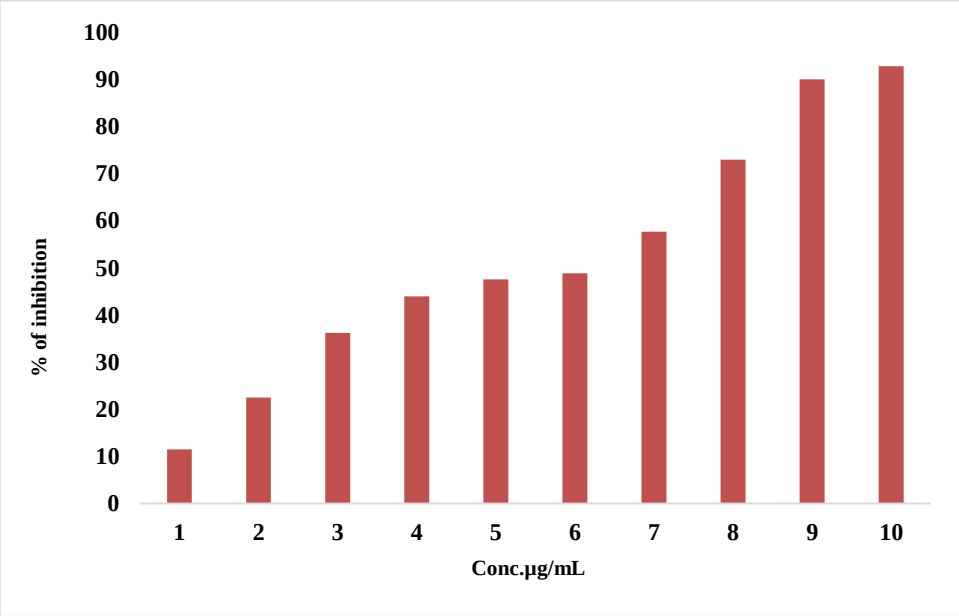

Fig.2b. DPPH activity of standard (Quercetin)

3.3.2. *ABTS Radical Scavenging Assay*

The relative antioxidant scavenging ability of the plant extracts to scavenge the $ABTS^{+}$ was compared with standard ascorbic acid (Table 4). The ABTS radical scavenging activity was highest in methanol extract, showed IC_{50} value of 58.98 µg/mL. The hexane extract showed IC_{50} value of 79.80μg/mL and aqueous extract showed 80.00 µg/mL. The hexane extract and aqueous extract did not produce much deviation in its inhibition activity, its 50 percent inhibition concentrations were very close to each other (Fig. 3). The $ABTS^{+}$ is reactive towards most antioxidants including phenols, thiols and vitamin C. The phenolic compounds present in the extracts act as an antioxidants directly through the mechanism of the reduction of oxidized intermediate in the chain reaction (Walker and Everette, 2009). Free radical (ABTS+) scavenging of *Cyanthillium cinereum* extracts might be due to the presence of high molecular weight phenolics. Lalhminghlui and Ganesh, (2018) in their study reported *Schima wallichii* scavenged ABTS free radicals.

Table 4. Antioxidant Activity by ABTS assay

Conc. (µg/mL)	% of Inhibition		
	Methanol extract	Hexane extract	Aqueous extract
10	17.99	6.76	12.5
20	21.79	8.52	16.25
30	23.87	11.02	18.75
40	29.06	33.08	20.78
50	45.32	41.10	23.27
60	50.86	43.60	27.32
70	53.63	47.36	30.93
80	56.74	50.12	43.75
90	65.05	72.43	56.35
100	67.82	78.69	72.50
IC_{50} value	**58.98**	**79.80**	**80.00**

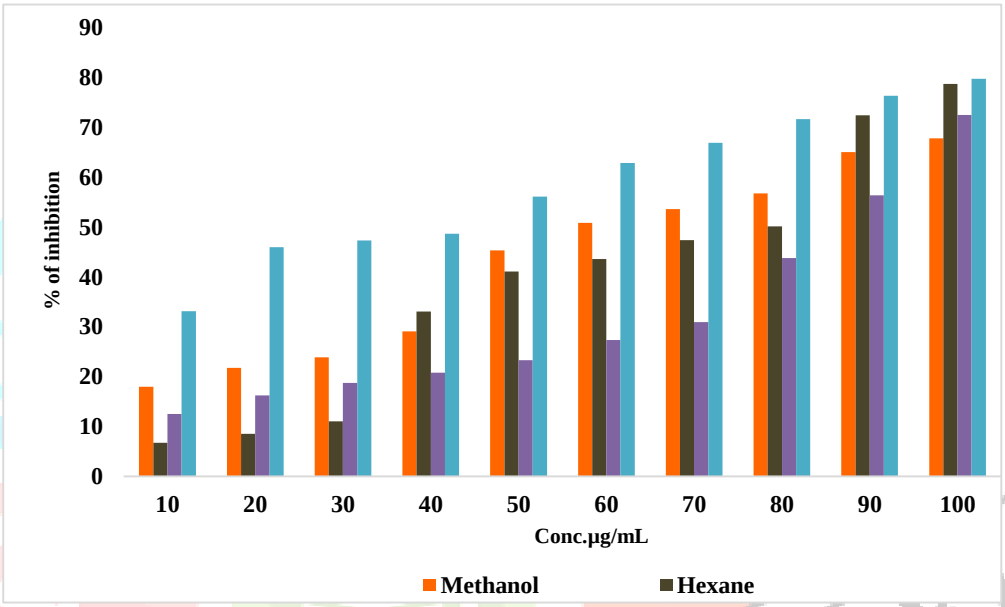

Fig. 3. ABTS assay of different extracts of *C. cinerium*

3.3.3. Phosphomolybdenum Assay

The basic principle of phosphomolybdenum activity depends on the reduction of Mo (IV) to Mo (V) by the plant extract which possess antioxidant potential compounds present in it. From the results obtained, methanol, hexane and aqueous extracts of showed maximum reduction potential of 1.211 µg/mL, 0.080 µg/mL and 0.690 µg/mL at 100 µg/mL and minimum reduction potential of 0.154 µg/mL, 0.023 µg/mL and 0.182 µg/mL at 10 µg/mL concentration respectively, when compared with that of the standard ascorbic acid (Table 5) (Fig. 4). The results suggested that the methanol extract of showed maximum effective antioxidant potential against the reduction of Mo (IV) to Mo (V). Recent investigation has shown that many flavonoids and related polyphenol contribute significantly to the antioxidant activity. The results suggested that the strong antioxidant activity of extracts might be due to the presence of phenolic compounds present in the extract (Falleh *et al*., 2005). Lalhminghlui and Ganesh, (2018) in their study reported that *Schima wallichii* plant had antioxidant potential.

Table 5. Antioxidant Activity by Phosphomolybdenum assay

Conc. (µg/mL)	Absorbance at 695nm		
	Methanol extract	Hexane extract	Aqueous extract
10	0.154	0.023	0.182
20	0.309	0.030	0.236
30	0.499	0.032	0.283
40	0.616	0.036	0.290
50	0.690	0.039	0.314
60	0.794	0.042	0.383
70	0.864	0.045	0.420
80	0.963	0.050	0.423
90	1.124	0.069	0.502
100	1.211	0.080	0.690

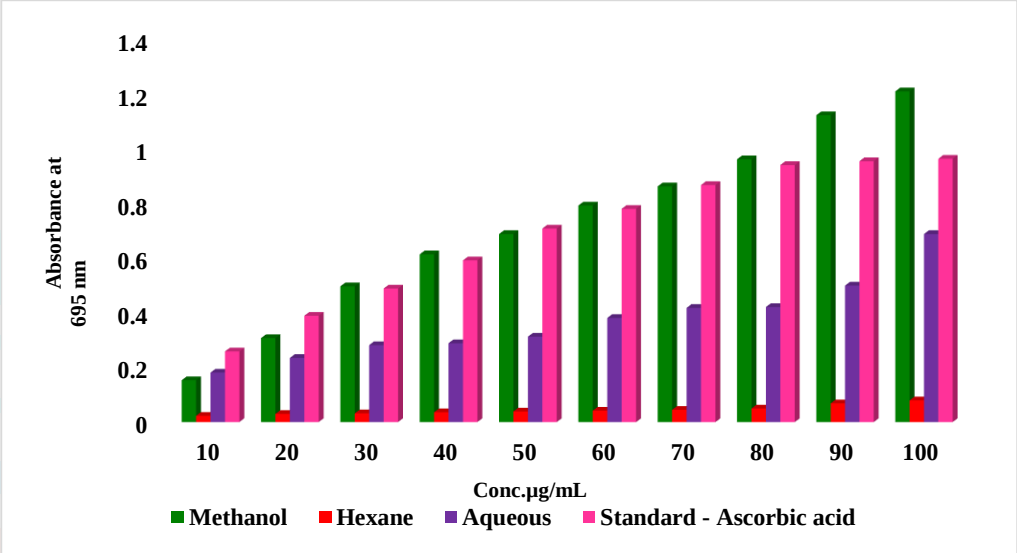

Fig. 4. Phosphomolybdenum assay of different extracts of *C. cinerium*

3.3.4. Ion Chelating Assay

The ferrous ion chelating assay was performed for all the three solvent extracts such as methanol, hexane and aqueous. All the three extracts showed active metal chelating properties. The highest percentage of chelating activity was produced by aqueous extract IC_{50} value of 46.87 µg/mL compared against the standard Na_2EDTA showed activity with IC_{50} value of 41.87 µg/mL. The methanol extract showed least ferrous ion chelating activity with IC_{50} value of 76.54 µg/mL. The hexane extract showed IC_{50} value of 69.74 μg/mL chelating activity (Table 6) (Fig. 5). The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals (Aboul Enein *et al*. 2003). The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. Nuran *et al*. (2012) reported high chelating activity of methanol extract of the mushroom *P. eryngii* collected from different regions of the Tunceli province of Turkey.

Table 6. Antioxidant Activity by Ion Chelating Assay

Concentration (µg/mL)	% of Inhibition		
	Methanol extract	Hexane extract	Aqueous extract
10	1.00	18.49	14.6
20	18.09	27.16	24.00
30	20.10	28.67	36.01
40	23.11	35.47	49.33
50	24.62	40.00	53.33
60	28.14	46.03	60.04
70	30.15	47.54	66.66
80	52.26	57.35	69.53
90	58.79	70.94	73.33
100	64.82	81.13	82.66
IC_{50} value	**76.54**	**69.74**	**46.87**

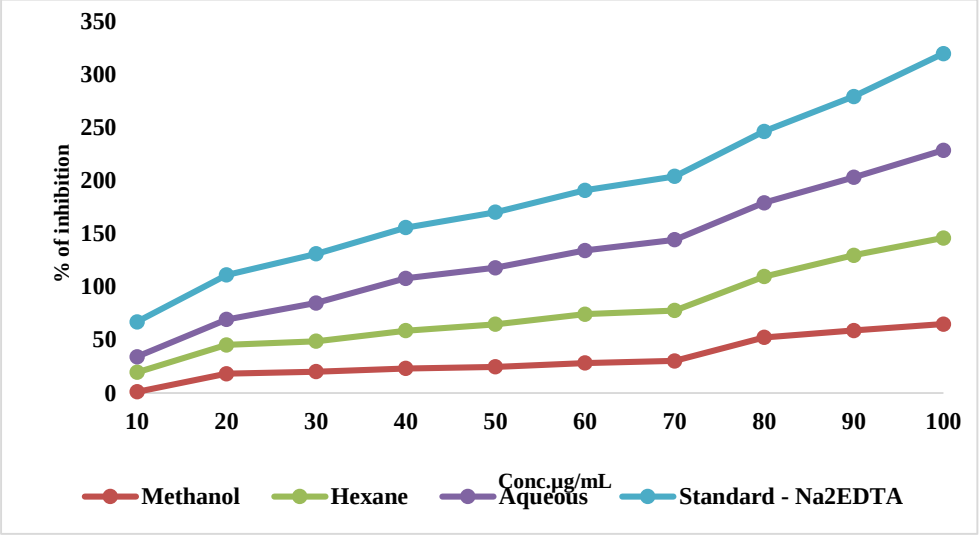

Fig. 5. Ion chelating activity of different extracts of *C. cinerium*

3.4. Antimicrobial Activity

The results for the analysis of antimicrobial potential of *Cyanthillium cinereum (L.)* H. Rob., using methanol extract, hexane extract and aqueous extract in agar well diffusion method (Fig. 6, 7 and 8). The microbial inhibition activity was performed against five human pathogenic microbes, such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Methicillin resistant staphylococcus aureus and Candida albicans*. It was observed that the different solvent extracts showed varied effect on each test microbe. Maximum zone of antimicrobial inhibition (5 mm) and (3 mm) was exhibited by 150 µg/mL of methanol extract, against *Pseudomonas aeruginosa* and *Methicillin resistant staphylococcus aureus* respectively. The hexane extract 4 mm inhibition zone against *Candida albicans* at 150 µg/mL dosage concentration. A very less zone of inhibition was produced by aqueous extract for all the dosages applied. Standard antibiotic produced (3-11 mm) diameter zone of inhibition for the microbes tested. Antimicrobial and therapeutic properties of phytoconstituents are safe to human beings and are devoid of any side effects. In this study the whole plant extracts (methanol and hexane) of *C. cinereum* exhibited excellent antimicrobial activity against all the five pathogens studied. From the results obtained its evident, that the plant contains essential phytochemicals responsible for the growth inhibition of bacterial and fungal metabolism together. Suja *et al*., (2019) in a study reported that antimicrobial activity of methanol extract of *Cyanthillium cinereum* produced efficient zone of inhibition against *Staphylococcus aureus* (19mm) and *Escherichia coli* (21mm).

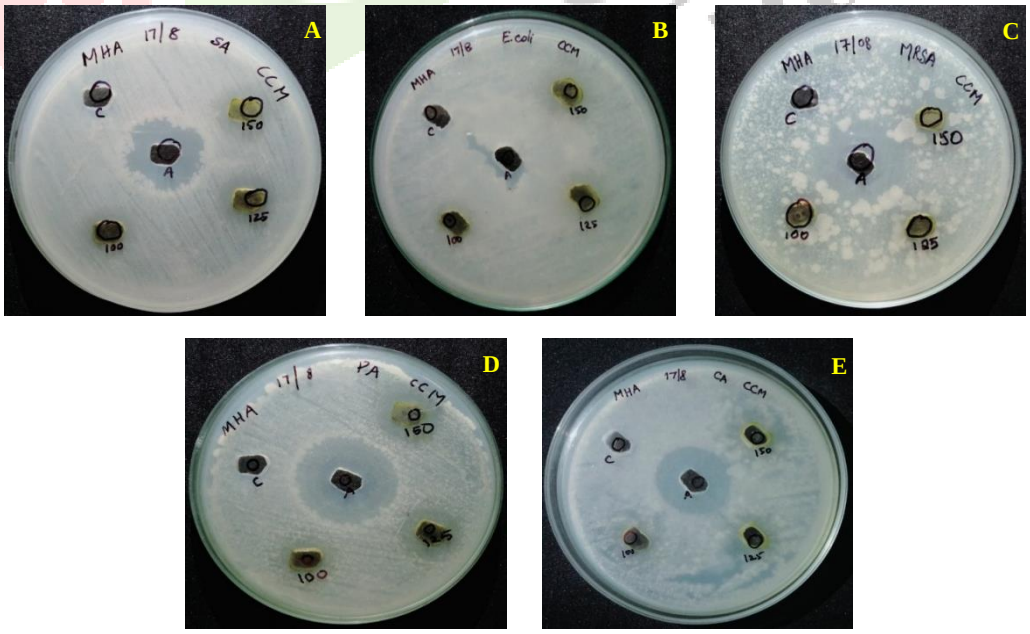

Fig.6. Antimicrobial Activity of Methanol Extract of *Cyanthillium cinerium* (L.) H. Rob.
(A) *Pseudomonas aeruginosa*; (B) *Staphylococcus aureus*; (C) *Escherichia coli*; (D) *Methicillin resistant Staphylococcus aureus* and (E) *Candida albicans*

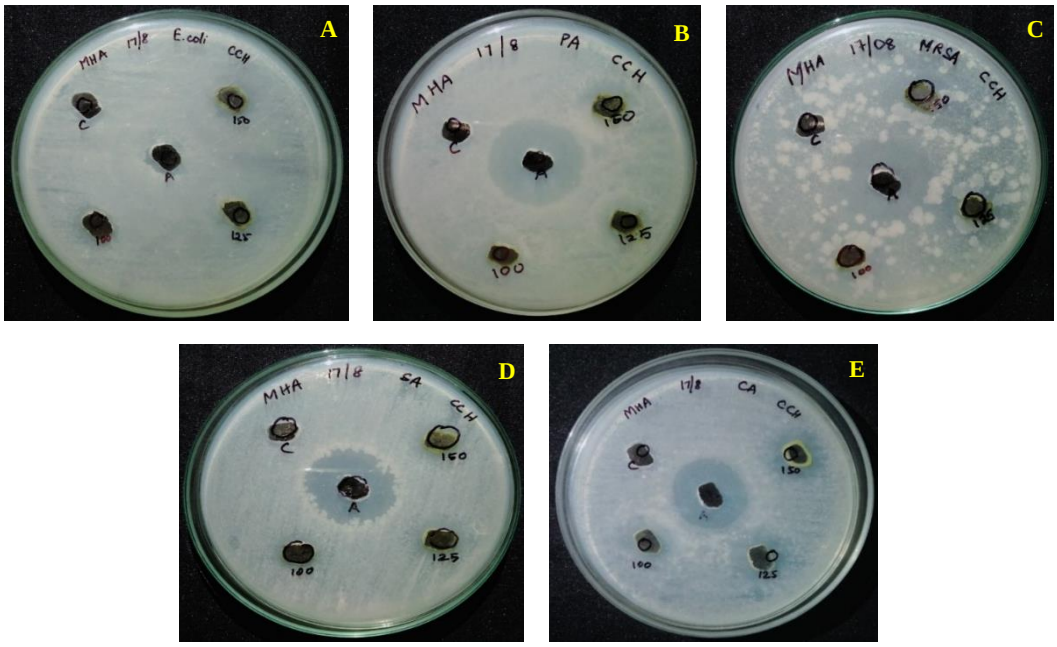

Fig.7. Antimicrobial Activity of Hexane Extract of *Cyanthillium cinerium* (L.) H. Rob. (A) *Pseudomonas aeruginosa*; (B) *Staphylococcus aureus*; (C) *Escherichia coli*; (D) *Methicillin resistant Staphylococcus aureus* and (E) *Candida albicans*

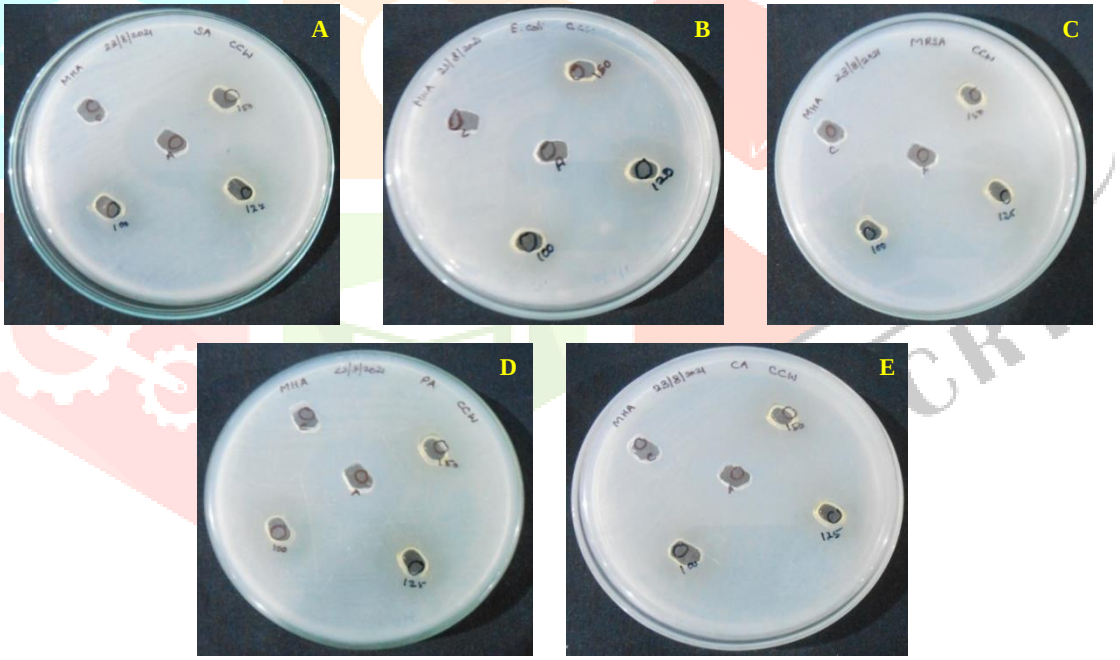

Fig.8. Antimicrobial Activity of Aqueous Extract of *Cyanthillium cinerium* (L.) H. Rob. (A) *Pseudomonas aeruginosa*; (B) *Staphylococcus aureus*; (C) *Escherichia coli*; (D) *Methicillin resistant staphylococcus aureus* and (E) *Candida albicans*

IV. CONCLUSION

In this study *Cyanthillium cinereum* whole plant extracts obtained with methanol, aqueous and hexane solvents were analysed for its potential antioxidant and antimicrobial activities. The extracts screened for preliminary phytochemicals showed the presence of alkaloids, carbohydrates, saponin and phenolic compounds. The extracts obtained with the polar and non polar solvents had high free radical scavenging activities compared with their respective standards. From the results, the study concluded that the whole plant methanol extract had therapeutic potential with antimicrobial efficiency against pathogenic micro organisms.

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