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AMELIORATIVE ANTIPYRETIC AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF LITTLE IRONWEED CYANTHILLIUM CINEREUM (L.) H. ROB.

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Abstract: In this study, the ameliorative antipyretic and antioxidant potential of methanolic extract of Cyanthillium cinerium (L.) H. Rob. leaf extract (MECC) was investigated**. M**ethanol 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 methanolic extracts. The in vivo antipyretic activity was determined by brewer's yeast induced pyrexia method. The quantity of total phenolic and flavonoid content was found to be in methanol extract 76.59 mg/g and 31.67 mg/g. 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 44.35 µg/mL and ABTS % inhibition was found to be IC50 3.52 µg/mL. The free radical scavenging potential of methanol extract with the phosphomolybdenum assay showed highest absorbance of 0.794nm at 60 µg/mLThe antipyretic result showed that the methanolic extract of Cyanthillium cinereum are non-toxic and possess significant antipyretic effect which may attribute in the presence of flavonoid and saponin in the extract.

Key words - Phytochemical Analysis, DPPH, ABTS, Free Radical Scavenging, Anti Pyretic Activity.

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 extensively found throughout the world's tropical and subtropical climates. 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. Additionally, it may be used as a treatment for impotency, asthma, cancer, cholera, colic pain, cough, diarrhea, and dysentery. (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.

Certain phytocompounds, like phenolic, vitamin, amino acid, and mineral compounds, may be employed as possible treatments for chronic illnesses like cancer and the heart. Methanol is commonly used for phenolic compounds extraction. 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).

The final step in preliminary validation of medicinal drugs involves induction of disease or disease−like conditions in suitable model animals and use of the extracted compounds to ascertain their effect on treatment. To validate the antipyretic effects of plant derived medicinal compounds, the brewer's yeast induced pyrexia has been extensively used (Alam et al., 2008).

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

II. MATERIALS AND METHODS

2.1. Plant material

The healthy leaves of Cyanthillium cinereum (L) H. Rob. Belongs to the family Asteraceae were collected from the roadsides of Nagpur, Maharashtra, India. In this study we use the leaf of Cyanthillium cinereum and it was Authenticated by Kajal Bondre, Assistant Professor, Department Of Pharmacognosy, Adarsh Institute Of Pharmacy, Nagpur, Maharashtra, India (Fig.1. a & b). Fresh, healthy leaves 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. Leaves of Cyanthillium cinerium

2.2. Drugs and chemical

All the chemicals used were of analytical grades, obtained from commercial suppliers. Paracetamol was obtained as gift sample from Mepro Pharmaceuticals Pvt. Ltd., Surendranagar, Gujarat, India. Brewer's yeast (Sigma-Aldrich). Double distilled water from all-glass still was employed throughout the study.

2.3. Preparation of plant extracts

leaves of the Cyanthillium cinereum was shade dried and powdered by a mechanical grinder. The dried powder was extracted with ethanol as a solvent by using Soxhlet apparatus. The powder (100 gm) was taken and placed in thimble made up of filter paper and inserted into the wide central tube of an extractor. Methanol is placed in the round bottom flask and brought to its boiling point up to 78 ^{O}C for 6-7 hours. Its vapors passed through the larger right-hand tube into the upper part of an extractor and then to the condenser. During this period, the active constituents were extracted, when the level of the extract reaches the top of the syphon tube. The process was continued until the drug was completely extracted, then the extract processed for evaporation. After the evaporation, the semi-solid jelly is formed. The plant extract was dark green in color and soluble in distilled water (Rob et al., 2022).

2.4. 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).

2.4.1. 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.

2.4.2. 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.

2.4.3. 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 H2SO4 concentrated. A grey color formed which showed the entity of terpenoids.

2.4.4. Tests for Glycosides: A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl3 mixture was mixed with the 10 ml aqueous plant extract and 1 ml H2SO4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

2.4.5. 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.

2.4.6. 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.

2.4.7. 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.

2.4.8. 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.

2.4.9. 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.

2.5. Quantitative phytochemical analysis

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

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

The plant powder (2 g) was soaked in different solvents such as methanol 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% Na2CO3 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)

2.5.2. 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,

$$X = [A.M0/A0.M]$$

where, A= absorption of sample, A0= absorption of standard (Paracetamol),
M= weight of sample (mg/mL) and M0= weight of quercetin in solution (mg/mL)

2.6. In vitro-antioxidant assay

2.6.1. 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

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

2.6.2. 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,

$$\text{ABTS·+ scavenging effect (\%)} = \{[AB - AA]/\ AB\} \times 100$$

where, AB is absorbance of ABTS radical + methanol;
AA is absorbance of ABTS radical + sample extract/standard.

2.6.3. 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).

2.6.4. 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. (Dinis et al. 1994).

2.7. Experimental animals

The animals were used as healthy male Wistar rats (200-250 g) were obtained Kusum life science A 3, MIDC, Wasmat, Hingoli. The animals were kept in sterilized cages in rooms at approximately 24±1℃ temperature and humidity of 55± 5% with a 12/12-hour light-dark cycle. Free access to food and water was allowed. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for Control and Supervision of Experiments on Animals. The study was approved by Institutional Animal Ethics Committee (IAEC) Rats were randomly divided into six groups with each group having 6 animals and received the following treatments.

2.7.1. Antipyretic activity study

Brewer's yeast induced pyrexia in rats

This antipyretic activity animal model was slightly modified method described by Adams et al. Antipyretic activity on Wistar rats was screened with Brewer's yeast induced pyrexia. The rats were divided into five groups of six each. The basal rectal temperature of the rats was measured by introducing 1-2 cm of digital thermometer in rectum. After measuring the basal rectal temperature, the pyrexia was induced by intraperitoneal injection, 20% suspension of brewer's yeast in normal saline at a dose of 10 ml/kg of body weight (Kumar et al., 2015). After 18 hrs of yeast injection, rats which showed a raise in temperature of at least 1°C were taken for the study. Immediately after 18 hrs of yeast injection (Devi et al., 2013). Animals in the various groups were treated as follows:

Control: 0.9 % normal saline administered by p.o for 24 hrs
Fever group: 10 ml/Kg (subcutaneous) of 20% suspension of brewer's yeast in normal saline.
Standard: Treatment of 150 mg/kg of paracetamol in saline by oral route
MECC 200 mg/kg: Treatment of 200 mg/kg MECC by oral route
MECC 400 mg/kg: Treatment of 400 mg/kg MECC by oral route
The rectal temperature was measured at 1-4 hrs after treatment.

2.8. Statistical analysis

The data were expressed as a mean ± standard deviation for 8 groups of six rats each. The data were subjected to statistical analysis using ANOVA followed by Dunnett's test to draw a comparison between control and treatment groups. $p \leq 0.05$ was considered as statistically significant.

III. RESULTS

3.1. Phytochemical Constituents of the Crude Extract

According to the qualitative phytochemical screening study, the crude extract of the of Cyanthillium cinereum extract was found to be positive for the presence of carbohydrate, alkaloids, saponin, phenol and glycosides, flavonoids, proteins, amino acids, triterpenoids were absent in methanolic extract of Cyanthillium cinereum (Table 1).

Table 1: Phytochemical constituent of Cyanthillium cinereum extract

Secondary metabolite	Test results
Carbohydrate	+
Glycosides	-
Proteins	-
Amino acids	-
Saponin	+
Flavonoids	-
Alkaloids	+
Triterpenoids	-
Phenol	+

+: Present, -: Absent

3.2. Quantitative Phytochemical Analysis

3.2.1. Total Phenolic content determination

The results of total phenolic content in the methanol extract at 650 nm absorbance was determined (Table 2, Fig 2). The total phenolic content in MECC was found to be 76.59 mg/g of extract calculated as paracetamol equivalent.

Table 2: Estimation of total phenolic content of MECC

Sample	Concentration (µg/ml)	Absorbance at 650 nm	
Standard (Paracetamol)	5	0.060	
	10	0.108	
	20	0.195	
	40	0.316	
	80	0.395	
	100	0.465	
Sample (MECC)	100	0.395	

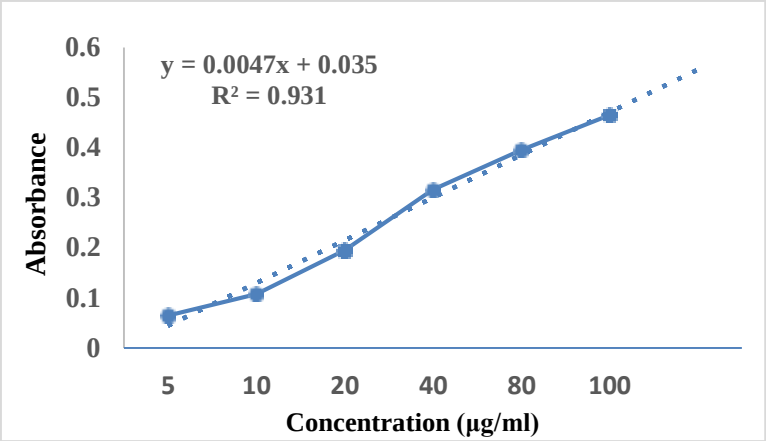

Figure 2: Standard graph for paracetamol for the estimation of total phenolic content

3.2.2. Total Flavonoid Content Of MECC

The results of total phenolic content in the methanol extract at 510 nm absorbance was determined (Table 3, Fig 3). The total flavonoid content in MECC was found to be 31.67 mg/g of extract calculated as paracetamol equivalent.

Table 3: Estimation of total flavonoid content of MECC

Sample	Concentration (µg/ml)	Absorbance at 650 nm
Standard (Paracetamol)	5	0.125
	10	0.230
	20	0.405
	40	0.665
	80	0.965
	100	1.125
Sample (MECC)	100	0.658

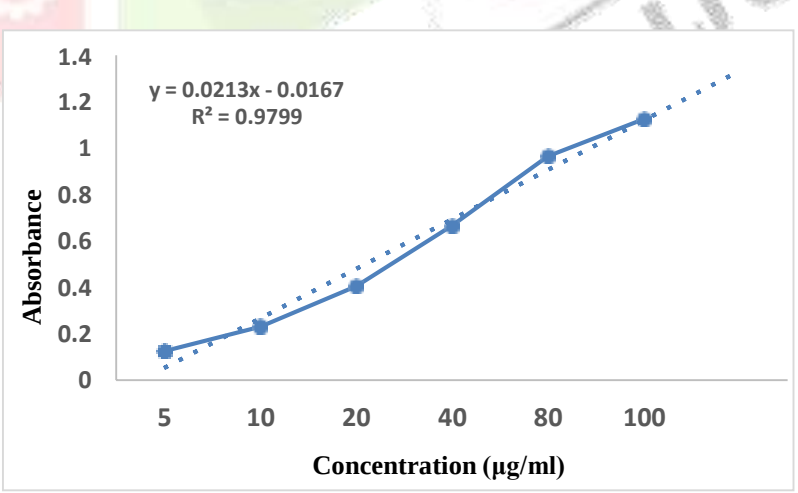

Figure 3: Standard graph of paracetamol for the estimation of total flavonoid content

3.3. In Vitro Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

Antioxidant activity of the samples was calculated through DPPH assay and reducing power assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Table-4 & Fig-4.

Table no 4: DPPH radical by various concentrations of test compound and Paracetamol

Concentrations (µg/ml)	Methanolic extract of Cyanthillium cinereum (MECC) (% Inhibition)	Paracetamol (% Inhibition)
5	23.55	30.85
10	30.25	36.15
20	38.28	42.65
40	48.45	58.90
80	52.30	72.05
160	60.95	85.65
IC50 value	**44.35**	**27.23**

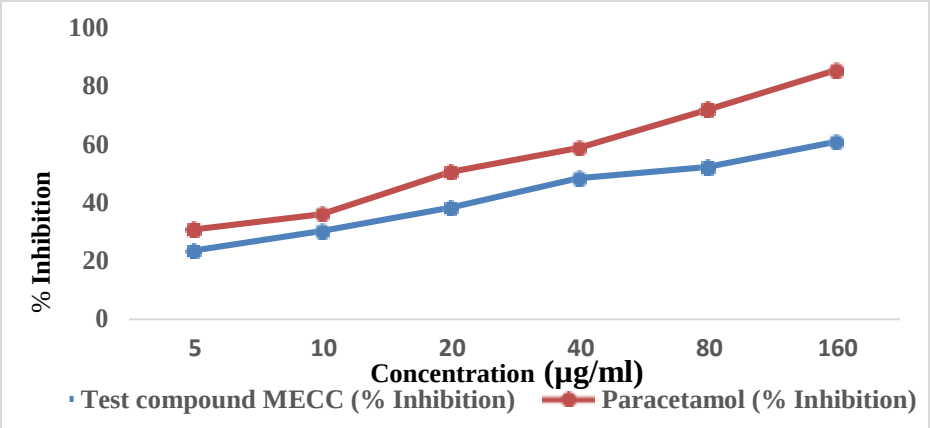

Figure 4: In vitro concentration dependent percentage inhibition of DPPH radical

3.3.2. ABTS Radical Scavenging Assay

The relative antioxidant scavenging ability of the plant extracts to scavenge the ABTS+ was compared with standard paracetamol (Table 5, Fig. 5).

Table 5: ABTS radical by various concentrations of test compound and Paracetamol

Concentrations (µg/ml)	Methanolic extract of Cyanthillium cinereum (MECC) (% Inhibition)	Paracetamol (% Inhibition)
0.25	25.60	42.56
0.5	32.65	50.34
0.75	53.25	65.12
1.0	69.7	79.84
1.25	77.4	90.23
1.5	85.9	96.41
IC50 value	**3.52**	**0.459**

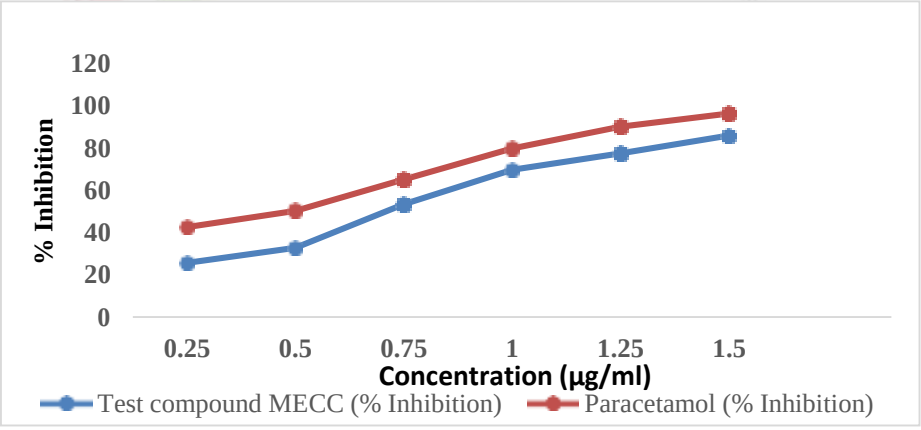

Figure 5: In vitro concentration dependent percentage inhibition of ABTS Radical

3.3.3. Phospho molybdenum 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. (Table 6 and figure 6). The basic principle of phosphor molybdenum activity depends on the reduction of Mo (IV) to Mo (V) by the plant extract which possess antioxidant potential compounds present in it.

Table no 6: Phosphomolybdenum assay by various concentrations of test compound and Paracetamol

Concentrations (µg/ml)	Methanolic extract of Cyanthillium cinereum (MECC) (% Inhibition)	Paracetamol (% Inhibition)
10	0.154	0.250
20	0.309	0.420
30	0.499	0.555
40	0.616	0.710
50	0.690	0.810
60	0.794	0.953

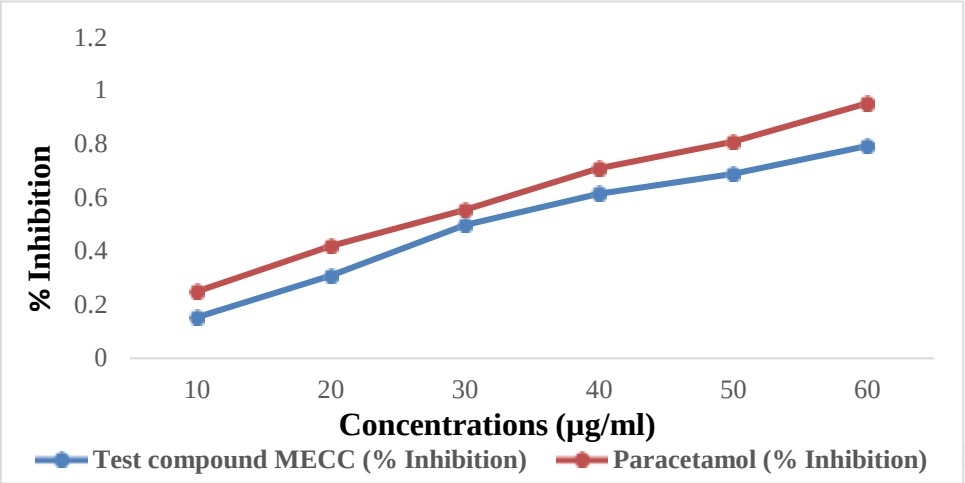

Figure 6: In vitro concentration dependent percentage inhibition of Phosphomolybdenum

3.3.4. Ion Chelating Assay

The ferrous ion chelating assay was performed for all the three solvent extracts such as methanol. All the three extracts showed active metal chelating properties. (Table 9) (Fig. 15).

Table no 7: Ion Chelating Assay by various concentrations of test compound and Paracetamol

Concentrations (µg/ml)	Methanolic extract of Cyanthillium cinereum (MECC) (% Inhibition)	Paracetamol (% Inhibition)
10	5.00	18.49
20	20.10	27.16
30	28.56	46.03
40	40.25	70.15
50	55.85	83.23
60	64.82	91.43
IC50 value	**76.54**	**46.87**

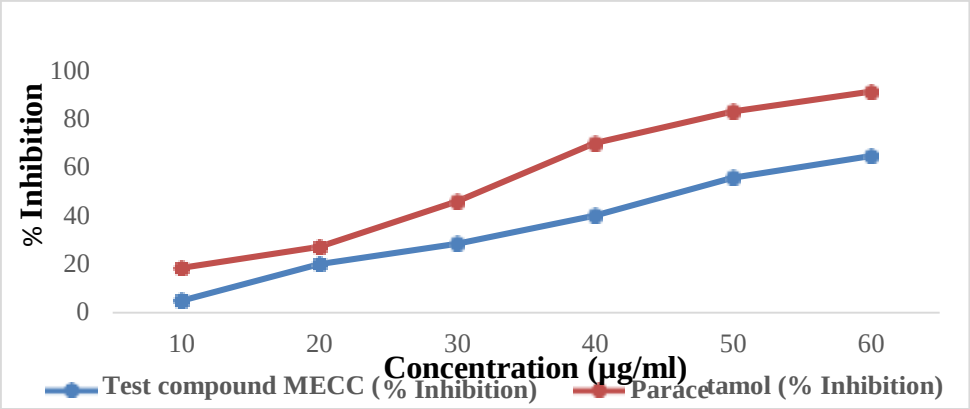

Figure 7: In vitro concentration dependent percentage inhibition of Ion Chelating Assay

3.4. Screening Of Antipyretic Activity

3.4.1. Brewer's Yeast Induced Pyrexia Model

The results of the antipyretic effects of aspirin and K. galanga extract were presented in Table 10 and Figure 16.

Table 8: Effect of plant extracts against Brewer's yeast induced Pyrexia in Wistar rats

Groups	Initial rectal temperature (°C)	Rectal temperature (°C) 18 hrs after Brewer's yeast induction	Rectal temperature (°C) after treatment with extract			
			1 hr	2 hr	3 hr	4 hr
Control	36.70 ± 0.13	37.9 ± 0.11	37.85 ± 0.04	37.75 ± 0.36	37.71 ± 0.08	37.62 ± 0.08
Fever Group	36.72 ± 0.08	39.15 ± 0.25	39.21 ± 0.22	39.28 ± 0.14	39.35 ± 0.12	39.42 ± 0.05
Standard	36.68 ± 0.10	39.20 ± 0.14	38.58 ±0.26	38.05 ± 0.10	37.85 ± 0.09	37.25 ± 0.09
MECC 200 mg/kg	36.73 ± 0.12	39.32 ± 0.29	39.07 ± 0.18	37.57 ± 0.13	37.25 ± 0.11	36.97 ± 0.10
MECC 400 mg/kg	36.77 ± 0.11	39.35 ± 0.33	38.93 ± 0.39	37.47 ± 0.08	37.32± 0.12	36.85 ± 0.07

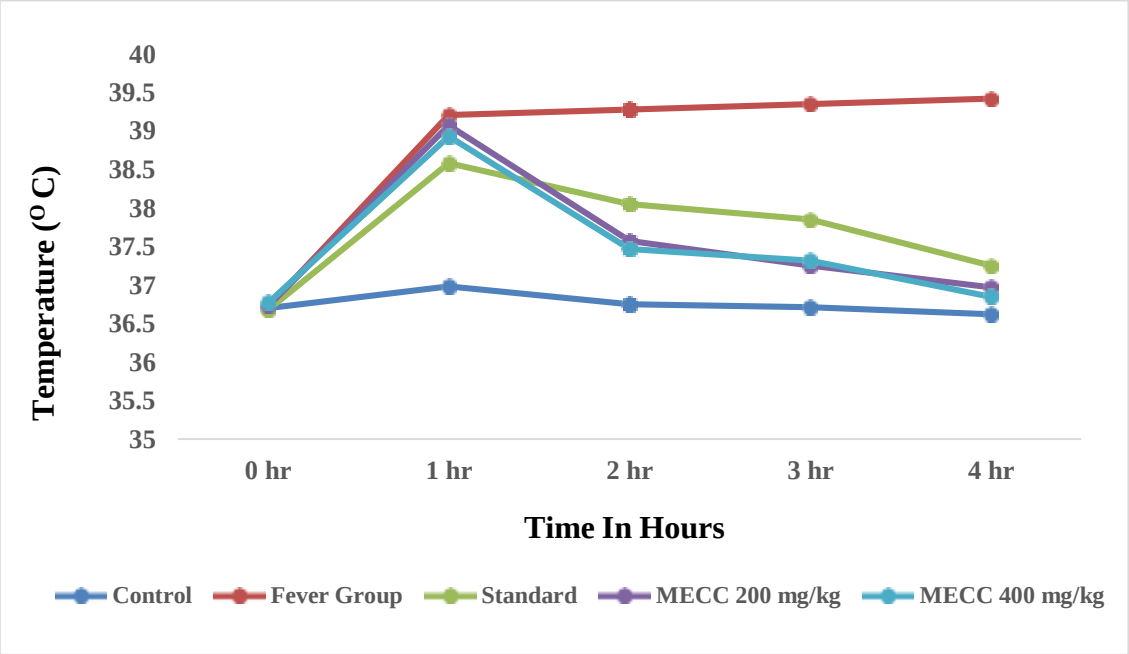

Figure 8: Anti-pyretic effect of Paracetamol and MECC in Brewer's yeast induced pyrexia. Each point shows reduction of rectal temperature after 0, 1, 2, 3 and 4 of treatment.

Administration of the brewer's yeast to the rat produced an increase in rectal temperature 18 hours after the yeast injection. Paracetamol at the dose of 150 mg/kg, caused a significant decrease in rectal temperature. The decrease in rectal temperature

began at 1 h after the paracetamol administration and continued up to 4 h. Cyanthillium cinereum at doses of 200 and 400 mg/kg orally did not decrease the rectal temperature induced by the brewer's yeast injection at all the time intervals.

3.4.2. Effect of plant extracts on body weight against Brewer's yeast induced Pyrexia

Table 9: Effect of plant extracts body weight

Animal Group	Body weight (g)
Control	230.2 ± 1.63
Fever Group	202.3 ± 4.63
Standard	225.17 ± 7.19***
MECC 200 mg/kg	210.33 ± 5.28*
MECC 400 mg/kg	220.52 ± 6.12***

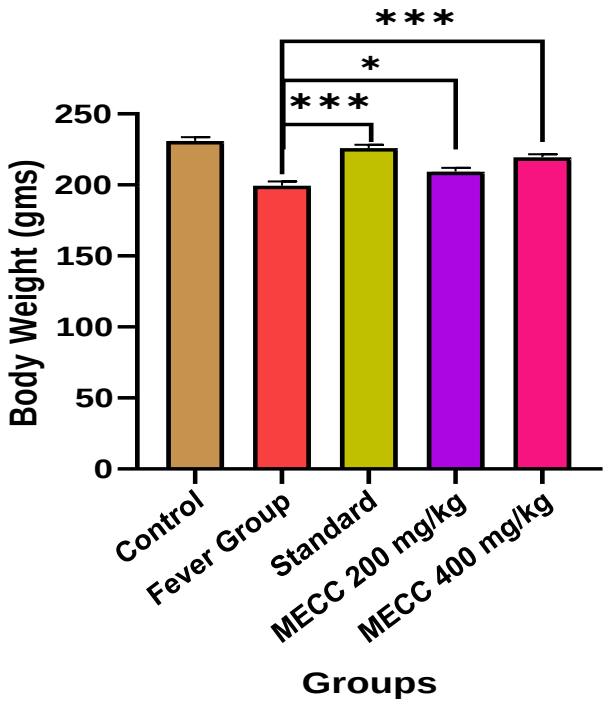

Figure 9: Body weight against Brewer's yeast induced Pyrexia

All values are represented as mean ± SEM, n=6 animals in each group. Data were analyzed by one way ANOVA, followed by Tukey's Multiple Comparisons ∗P < 0.05, ∗∗P < 0.01, ∗∗∗P < 0.001 compared with fever group.

IV. DISCUSSION

The current study deals with the inquiry of the effects of Cyanthillium cinereum in animal models of brewer's yeast induced pyrexia. In these study shows the qualitative phytochemical screening study, the crude extract of the of Cyanthillium cinereum extract was found to be positive for the presence of carbohydrate, alkaloids, saponin, phenol and glycosides, flavonoids, proteins, amino acids, triterpenoids were absent in methanolic extract of Cyanthillium cinereum (Saini et al., 2012).

The total phenolic content in MECC was found to be 76.59 mg/g of extract calculated as paracetamol equivalent (Azim et al., 2021). The total flavonoid content in MECC was found to be 31.67 mg/g of extract calculated as paracetamol equivalent.

The DPPH free radical scavenging activity of the extract was estimated by comparing the percent inhibition of MECC with standard paracetamol. The IC50 value of extract and paracetamol was found to be 44.35 and 27.23 respectively (Nigussie et al., 2021). The result showed that the extract has hydrogen donating ability and serve as free radical scavenger. The activity was found to be increased in a dose dependent manner. IC50 value of paracetamol and MECC was found to be 0.459 and 3.52 respectively.

Phosphomolybdenum activity of methanol extracts of showed maximum reduction potential of 0.794 µg/mL, at 60 µg/mL and minimum reduction potential of 0.154 µg/mL at 10 µg/mL concentration respectively, when compared with that of the standard paracetamol. The ferrous ion chelating assay produced by methanolic extract IC50 value of 76.54 µg/mL compared against the standard paracetamol showed activity with IC50 value of 46.87 µg/mL.

The present results showed that MECC possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug) from 1 h to 4 h. So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action of MECC as that of paracetamol. Inhibition of any of these mediators may bring about antipyretic effect.

V. CONCLUSION

On the basis of the study the formulation showed significant in vitro anti-oxidant activity by terminating the actions of free radicals. The MECC (200 mg/kg and 400 mg/kg) was studied for its antipyretic activity. Paracetamol (150 mg/kg) was used as standard for the rat models. The antipyretic activity was evaluated by Brewer's yeast induced pyrexia model in which paracetamol (150 mg/kg) was used as standard. In antipyretic activity, MECC demonstrated significant ($P < 0.05$) protection by reducing yeast evoked elevated body temperature which is comparable to standard drug. Antibacterial activity was evaluated via disk diffusion method. The potential activity of extract may be due to the presence of phenols, flavonoids and other phytochemical constituents present in it.

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