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EVALUATION OF *IN VITRO* ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *CYANTHILLIUM CINEREUM*

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ABSTRACT

Cyanthillium cinereum, commonly known as Vernonia cinerea or small ironweed, is a herbaceous plant in the Asteraceae family that is endemic to tropical and subtropical climates around the world. This study evaluates the *in vitro* antidiabetic activity of the ethanolic extract of *Cyanthillium cinereum*. The plant, traditionally used for various medicinal purposes, was analyzed for its phytochemical constituents, molecular docking interactions, and enzyme inhibition potential. Phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, saponins, and cardiac glycosides. Molecular docking studies revealed that luteolin, a bioactive flavonoid, exhibited strong binding affinity with α-glucosidase and α-amylase, key enzymes involved in carbohydrate metabolism. *In vitro* assays demonstrated that the ethanolic extract significantly inhibited α-glucosidase, showing a higher potency compared to the standard drug acarbose. However, its α-amylase inhibition was moderate in comparison to acarbose. These findings suggest that *Cyanthillium cinereum* has potential as a natural antidiabetic agent by modulating carbohydrate metabolism. Further studies, including clinical trials, are recommended to validate its efficacy and therapeutic applications.

Keywords: *Cyanthillium cinereum,* antidiabetic activity, phytochemistry, molecular docking, α-glucosidase, α-amylase.

INTRODUCTION

Diabetes mellitus is a group of disorders that affect how the body utilizes glucose, which is a crucial energy source for cells, muscles, and tissues. High blood sugar levels can lead to severe health complications. Type 1 diabetes, formerly known as juvenile-onset diabetes, is an autoimmune disease that specifically targets the pancreas, leading the body to attack its own insulin-producing cells[1]. Type 2 diabetes, which accounts for over 90% of diabetes cases, occurs when the body either does not use insulin effectively or does not produce enough. Gestational diabetes, on the other hand, occurs when blood sugar levels rise during pregnancy in women who previously had no history of diabetes. The causes of diabetes include insulin resistance, autoimmune diseases, hormonal imbalances, and pancreatic damage[2]. Common signs and symptoms include excessive thirst (polydipsia), excessive hunger (polyphagia), lethargy, blurred vision, weight loss, nausea, vomiting, abdominal pain, frequent urination (polyuria), and the presence of sugar in the urine (glycosuria). If left untreated, diabetes can lead to severe complications such as cardiovascular diseases, heart attacks, strokes, high blood pressure, diabetic neuropathy, diabetic retinopathy, diabetic nephropathy, and foot complications[3]. Antidiabetic agents used to manage diabetes include insulin, pramlintide, gliclazide, glimepiride, metformin, pioglitazone, and rosiglitazone[4].

Cyanthillium cinereum, also known as Vernonia cinerea or little ironweed, belongs to the Asteraceae family. This annual herb is native to tropical Africa, tropical Asia, India, Indochina, South America, and parts of North America, including Florida. It grows up to 120 cm tall and produces clusters of small, purple or blue flowers. The plant contains various phytochemicals such as cardiac glycosides, alkaloids, phenols, flavonoids, steroids, tannins, phlobatannins, and saponins, many of which have medicinal properties. Flavonoids and phenols, in particular, are powerful antioxidants that play a significant role in health care[5]. *Cyanthillium cinereum* is widely used in traditional medicine for its various therapeutic properties. It has been found to help people quit smoking, alleviate arthritis, muscle pain, and inflammation, and treat oral ulcers, sore throats, and gum infections. Additionally, it possesses antibacterial and antifungal properties, making it effective against infections. It is also known for its liver-supporting properties, helping to detoxify and protect the liver from oxidative stress and toxin-related damage[6].

Figure no.1: *Cyanthillium cinereum*

The plant, commonly referred to as little ironweed or common vernonia, is indigenous to tropical Asia, particularly in regions of India, Sri Lanka, Southeast Asia, and China. It has also been introduced to Africa, northern Australia, the Pacific Islands, and the Americas. It thrives in dry to damp forests, grasslands, roadsides, and riverbanks. Botanically, *Cyanthillium cinereum* is a fast-growing herbaceous plant that flourishes in tropical and subtropical climates. It typically grows between 30 and 100 cm tall, with slender, branching stems that are either purplish or green. Its leaves are lanceolate to elliptic, measuring between 4-10 cm in length and 1-4 cm in width. These alternately arranged leaves have finely serrated margins and a slightly hairy texture, allowing the plant to tolerate moderate dry conditions. The plant's inflorescence consists of small, attractive flower heads that bloom in late summer and fall, providing an important food source for pollinators like bees and butterflies. After flowering, it produces small, dry fruits with a tuft of silky hairs, facilitating wind-borne seed dispersal[7]. *Cyanthillium cinereum* has a fibrous root system that enables it to thrive in disturbed areas such as roadsides, open fields, and wastelands, where it often plays a role in soil rehabilitation. The plant is adaptable to various soil types and light conditions, capable of growing in poor, dry soils while tolerating full sun and moderate shade. Though not commonly cultivated for ornamental purposes, its resilience, rapid growth, and ability to support pollinators make it an ecologically valuable species. In traditional herbal medicine, it is valued for its anti-inflammatory and antibacterial properties, further contributing to its significance in natural healthcare systems[8].

MATERIALS AND METHODS

Collection and authentication of *Cyanthillium cinereum:*

Cyanthillium cinereum was collected from Thiruvananthapuram in the month of November. The plant material was authenticated by the Department of Botany, Christian College, Kattakada. The sample is then thoroughly cleaned with fresh water and air dried at room temperature in the shade for 15 days before being pulverized into coarse powder using a mechanical grinder. The powdered substance was stored in an airtight container[9,10].

Preparation of ethanolic plant extract of *Cyanthillium cinereum:*

The leaves of *Cyanthillium cinereum* were thoroughly washed under running tap water to remove any impurities and then dried under shade for 15 days. Once dried, they were pulverized into a coarse powder using a mechanical grinder. For the preparation of the ethanolic extract, 50g of the powdered material was subjected to maceration with 1000ml of 75% ethanol, with continuous shaking at room temperature to facilitate extraction. After one week, the extract was strained using muslin cloth to separate the liquid from the plant residue. The crude extract

was then concentrated to dryness under reduced pressure and controlled temperature, ensuring the preservation of its bioactive compounds. Finally, the dried extract was stored in an airtight container for further use[11].

$$\textbf{Percentage yield} = \frac{w2}{w1} \text{x} 100$$

Where,

W1-weight in grams of dried plant material, W2-weight in grams of extract obtained

Preliminary phytochemical screening

Qualitative phytochemical screening was carried out for flavonoids, tannins, cardiac glycosides, saponins, alkaloids, carbohydrate[12,13].

***IN SILICO* MOLECULAR DOCKING STUDIES**[14]

Step1: Ligand preparation

Chemical structure of the compounds was obtained from the world's largest freely accessible chemistry databases PubChem. Chemical structures of the compounds are downloaded in 3D format and converted to sdf format. Finally, the input format prepared as pdb ligand format and can be visualized in open babel.

Step 2: Protein preparation

Target molecules such as human arachidonate lipoxygenase and human cyclooxygenase COX 2 can be determined by literature survey and respective PDB files such as IC7V, IYPU were downloaded from protein data bank. Then the protein is downloaded in crystal structure and after processing convert this to input format.

Step 3: Protein processing

Protein structure processing is done by removal of water molecules, hydrogens, heterostoms and bound ligand if any.

Step 4: Docking analysis

Docking analysis was done by Auto dock Vina Program in PyRx software. For this purpose, installed Auto dock tools (Python 2.7.1, MGL tools 1.5.4 and Open babel) and PyRx. Installed Auto dock tools (Python 2.7.1, MGL tools 1.5.4 and Open babel) and PyRx.

Step 5: Molecular visualization

PyMOL is a software used for the protein preparation and molecular visualization. It produces high quality 3D images of protein.

***IN VITRO* STUDY FOR ANTIDIABETIC ACTIVITY**

Alpha glucosidase inhibition assay

Procedure

The effect of the sample on α-glucosidase activity was determined according to the method described by Shai et al., (2011) with slight modification. 400 µL of α-glucosidase (0.067 U/ml) was preincubated with different concentration of the sample for 30 min. Then 200 µL of 3.0 mM (pNPG) used as substrate dissolved in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-colored para- nitro phenol released from pNPG at 400 nm. The results were expressed as percentage of inhibition. Same procedure was done with Acarbose (1mg/ml stock) which was used as standard[15].

$$\textbf{Inhibitory activity (\%)} = \textbf{(B-T/B-C)} \times \textbf{100}$$

Where,
B is the absorbance of blank.
T is the absorbance in the presence of test substance.
C is the absorbance of control.

Alpha amylase inhibition assay

Procedure

The anti-diabetic activity of the test samples was determined according to the method described in the Worthington Enzyme Manual with slight modifications. In brief, 500µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/mL of α- amylase enzyme and different concentration of the test sample as enzyme inhibitor were pre-incubated at 37°C for 10 min. After the pre-incubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at room temperature for 5 mins. The reaction was stopped using 1.0 mL of dinitrosalicylic acid (DNSA) reagent. The test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. The volume of the reaction mixture was made up to 10mL by adding distilled water, and the absorbance was measured at 540 nm using UV-Visible spectrophotometer. The absorbance was compared with the controls and blank that contained buffer instead of test sample[16].

$$\textbf{Percentage inhibition} = \frac{(\mathbf{B} - \mathbf{A}) \times \mathbf{100}}{(\mathbf{B} - \mathbf{C})}$$

C- Absorbance of the Control with starch and without alpha amylase
B- Absorbance of the Control with starch and alpha amylase
A- Absorbance of the Test.

RESULTS AND DISCUSSION

Percentage yield

NAME OF PLANT	PLANT PART USED	METHOD OF EXTRACTION	SOLVENT USED FOR EXTRACTION	PERCENTAGE YIELD (% w/w)
Cyanthillium cinereum	Leaf, stem, flower	Maceration	Ethanol	23 % w/w

Table no:1 Percentage yield of plant extract of *Cyanthillium cinereum*

Preliminary phytochemical screening

SL NO	NAME OF THE CONSTITUENTS	NAME OF THE TEST	INFERENCE
1.	Flavonoids	Shinoda test	+
		Lead acetate test	+
		Alkaline reagent test	+
2.	Tannins	Ferric chloride test	+
3.	Cardiac glycosides	Legal test	+
4.	Saponins	Foam test	+
5.	Alkaloids	Dragendorff's test	+
6.	Carbohydrates	Fehling's test	+

(+) - Positive

(-) - Negative

Table 2: Summary of phytochemical screening of ethanolic plant extract of *Cyanthillium cinereum*

In silico docking studies of *Cyanthillium cinereum*

In silico docking studies predict interactions between ligands and target molecules. *Cyanthillium cinereum* contains luteolin, a flavonoid docked with α-glucosidase and α-amylase, key enzymes in carbohydrate metabolism. α-Amylase aids in starch breakdown, while α-glucosidase facilitates carbohydrate digestion. Inhibiting α-glucosidase slows digestion, preventing postprandial hyperglycemia, a major factor in diabetes. Luteolin shows good binding affinity with these proteins, suggesting potential antidiabetic activity

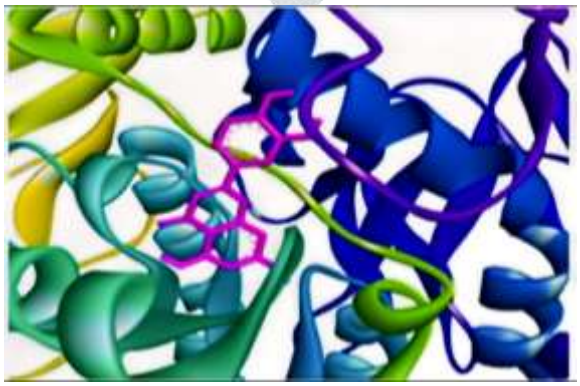

Figure no.3: Docking image of Luteolin with alpha glucosidase protein.

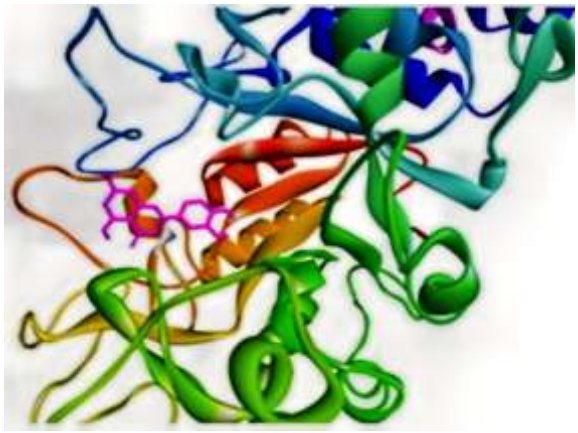

Figure no.4: Docking image of Luteolin with alpha amylase protein.

COMPOUND	RECEPTOR	NUMBER OF HYDROGEN BONDS	BINDING AFFINITY (kcal/mol)
Luteolin	Alpha amylase	3	-6.80

Table 3: Molecular docking value of Luteolin with alpha amylase

COMPOUND	RECEPTOR	NUMBER OF HYDROGEN BONDS	BINDING AFFINITY (kcal/mol)
Luteolin	Alpha glucosidase	3	-9.35

Table 4: Molecular docking value of Luteolin with alpha glucosidase

IN VITRO* STUDY OF *CYANTHILLIUM CINEREUM

Alpha glucosidase inhibition assay

The inhibitory rate of alpha glucosidase increases gradually with increasing concentration of EECC. The α-Glucosidase Inhibition Assay assesses a sample's capacity to inhibit the enzyme under certain conditions. The test entailed incubating the enzyme with different amounts of the material and measuring the absorbance of the yellow-colored para-nitrophenol at 400 nm. When comparing with standard drug acarbose(IC_{50} of 16.72 µg/mL), EECC have more alpha glucosidase inhibitory effect(IC_{50} of 11.24 µg/ml). When analyzing the result the EECC shows maximum α-Glucosidase inhibition at 100 µg/mL.

SAMPLE CODE	CONCENTRATION (mg/ml)	ABSORBANCE AT 400nm	PERCENTAGE INHIBITION (%)
Blank	-	1.493	
Control	-	0.024	
EECC	6.25	0.932	38.19
	12.5	0.698	54.11
	25	0.327	79.37
	50	0.131	92.72
	100	0.098	94.96
	IC 50*		11.24 µg/ml

Table 5: Effect of ethanolic plant extract of *Cyanthillium cinereum* in alpha glucosidase inhibition assay

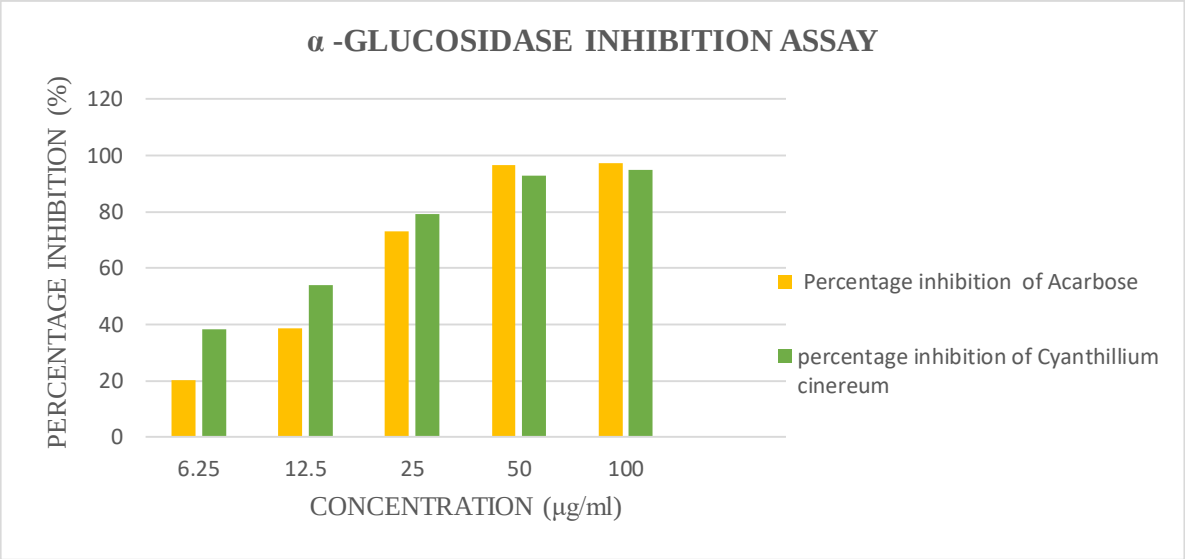

Figure no.5: Comparison of percentage inhibition of acarbose and ethanolic extract of *Cyanthillium cinereum*

Alpha amylase inhibition assay

The inhibitory rate of alpha amylase increases with increasing concentration. In α-Amylase Inhibition assay, the results for EECC shows that at a concentration of 100 µg/ml, the percentage inhibition was 57.94%, with an IC_{50} value of 78.47 µg/ml. When comparing with standard drug acarbose(IC_{50} of 36.44 µg/ml), EECC have moderate alpha amylase inhibitory effect(IC_{50} of 78.47 µg/ml). This result suggests that the EECC exhibited moderate inhibitory activity against α-amylase, indicating potential anti-diabetic properties but with lower potency compared to the standard Acarbose.

SAMPLE CODE	CONCENTRATION (mg/ml)	ABSORBANCE AT 540 nm	PERCENTAGE INHIBITION (%)
Blank	-	1.437	
Control	-	0.032	
EECC	6.25	1.411	1.85
	12.5	1.236	14.31
	25	1.025	29.32
	50	0.877	39.86
	100	0.623	57.94
	IC 50*		78.47 µg/ml

Table 6: Effect of ethanolic plant extract of *Cyanthillium cinereum* in alpha amylase inhibition assay

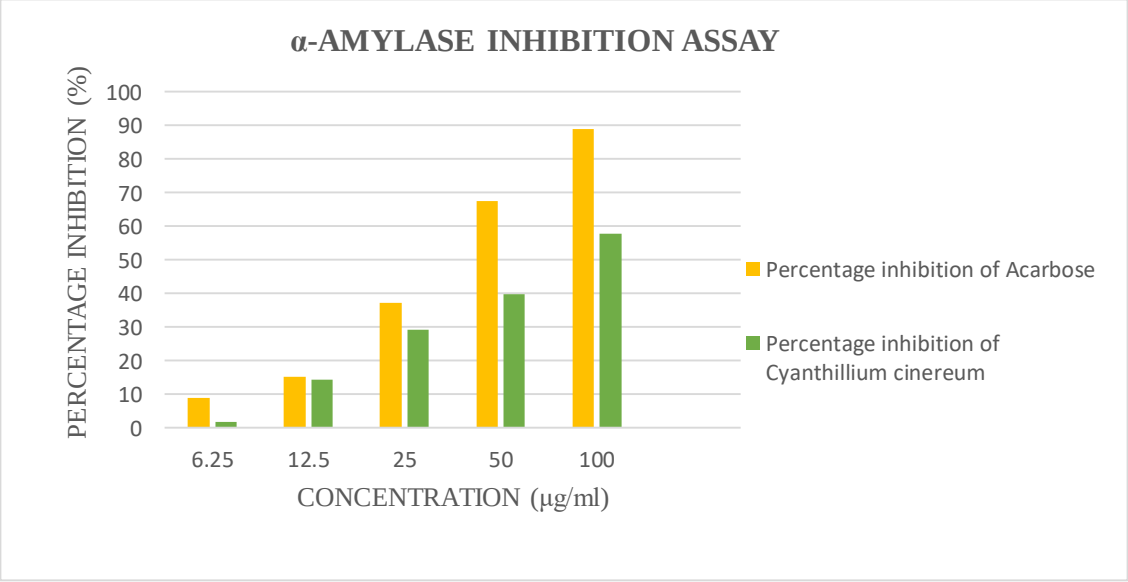

Figure no.6: Comparison of percentage inhibition of acarbose and ethanolic extract of *Cyanthillium cinereum*

DISCUSSION

Cyanthillium cinereum, also known as *Vernonia cinerea* or Little Ironweed, belongs to the Asteraceae family and has been traditionally used in Ayurvedic medicine for treating renal diseases, swelling, stomach pain, menstrual cramps, and as an antihelminthic. Phytochemical analysis of the plant reveals the presence of flavonoids, phenols, alkaloids, tannins, saponins, steroids, and cardiac glycosides, which are known for their antioxidant and medicinal properties. Research highlights its antioxidant, antibacterial, anticancer, anti-inflammatory, and smoking cessation properties. The whole plant was collected from Thiruvananthapuram in November, identified, and authenticated by the Department of Botany, Christian College, Kattakada. The sample was thoroughly washed, air-dried under shade for 15 days, and pulverized using a mechanical grinder. For the ethanolic extract, 50g of the powdered material was extracted with 1000ml of 75% ethanol, subjected to continuous shaking at room temperature, and concentrated to dryness under reduced pressure and controlled temperature before being stored in an airtight container. The percentage yield of the ethanolic extract was found to be 23% w/w. The freshly prepared extract was subjected to phytochemical screening, confirming the presence of flavonoids, tannins, cardiac glycosides, saponins, alkaloids, and carbohydrates.

In silico molecular docking studies were conducted to predict the affinity of ligands to receptor proteins. Docking is an economical, reliable, and time-saving method for screening lead molecules. A docking-based virtual approach was performed using AutoDock VINA through PyRx 0.8 tools, and PyMOL software was used for 3D visualization of macromolecules. Docking studies revealed that luteolin exhibited strong binding to both α-glucosidase and α-amylase proteins, suggesting its potential to inhibit these enzymes. Molecular docking experiments showed that luteolin had a stable binding affinity of -6.80 kcal/mol against α-amylase, while it exhibited the highest binding affinity of -9.35 kcal/mol against α-glucosidase, interacting with three hydrogen bonds.

To confirm the *in silico* results, *in vitro* studies were performed on α-amylase and α-glucosidase enzymes. The α-glucosidase inhibition assay was conducted to evaluate the antidiabetic potential of the ethanolic extract of *Cyanthillium cinereum*. Since α-glucosidase is responsible for carbohydrate digestion, inhibiting this enzyme delays glucose absorption, preventing postprandial hyperglycemia. The ethanolic extract was tested at various concentrations (6.25 - 100 µg/mL) and exhibited dose-dependent inhibition of α-glucosidase, with higher concentrations showing greater inhibitory effects. The IC_{50} value for EECC was found to be 11.24 µg/mL, indicating significant inhibitory potential, suggesting its use as an antidiabetic agent. Compared to Acarbose, EECC showed higher inhibition, with an IC_{50} value of 11.24 µg/mL. The α-amylase inhibition assay evaluated the ability of EECC to inhibit α-amylase, which breaks down starch into maltose and glucose. Inhibiting this enzyme slows carbohydrate digestion, reducing postprandial blood glucose spikes, making it an important target for diabetes management. The IC_{50} value of EECC for α-amylase inhibition was 78.47 µg/mL, whereas Acarbose had an IC_{50} value of 36.44 µg/mL. While EECC showed lower α-amylase inhibition compared to Acarbose, its inhibitory activity was still significant and dose-dependent, highlighting its potential as a natural antidiabetic agent.

CONCLUSION

The ethanolic extract of *Cyanthillium cinereum* was tested *in vitro* for its antidiabetic potential. Phytochemical research revealed the existence of bioactive substances with medicinal effects, including flavonoids, tannins, alkaloids, saponins, and cardiac glycosides. Molecular docking experiments revealed a high binding affinity of luteolin for alpha amylase and alpha glucosidase proteins, implying a potential involvement in glucose metabolism. The EECC efficiently inhibited α-glucosidase and α-amylase, indicating its promise as a natural antidiabetic drug. These data suggest that *Cyanthillium cinereum* is a viable choice for further investigation in diabetes control.

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