In Vitro Antidiabetic and Antioxidant Activity of Aerial Parts of *Coldenia procumbens* Linn. (*Boraginaceae*)

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Abstract: *Coldenia procumbens* Linn., a medicinal plant traditionally used in folk medicine, was evaluated for its in vitro antidiabetic and antioxidant activities using aerial parts. Methanolic and aqueous extracts were prepared and subjected to phytochemical screening, which revealed the presence of flavonoids, phenolics, saponins, tannins, and glycosides. Antidiabetic activity was assessed by α-amylase and α-glucosidase inhibitory assays, while antioxidant potential was evaluated using DPPH free radical scavenging assay, ABTS assay, and reducing power assay. The methanolic extract showed significant inhibition of α-amylase (up to 72.3% at 500 µg/mL) and α-glucosidase (68.7% at 500 µg/mL), comparable to the standard acarbose. In antioxidant assays, the extract demonstrated dose-dependent scavenging activity with IC_{50} values of 48.2 µg/mL for DPPH and 41.6 µg/mL for ABTS, indicating strong free radical neutralization ability. These results suggest that the aerial parts of *Coldenia procumbens* possess promising in vitro antidiabetic and antioxidant properties, likely attributable to its phytochemical constituents. Further in vivo studies and compound isolation are recommended to explore its therapeutic potential.

Keywords: *Coldenia procumbens*, Antidiabetic activity, Antioxidant activity, α-Amylase, DPPH assay, ABTS assay etc.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. It is a major global health concern, affecting over 500 million people worldwide, and is projected to become the seventh leading cause of death by 2030. Chronic hyperglycemia in diabetes is associated with long-term damage to vital organs, including the eyes, kidneys, nerves, heart, and blood vessels.[1]One of the key pathological mechanisms underlying diabetes and its complications is **oxidative stress**, a condition marked by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense system. Elevated glucose levels accelerate oxidative stress, which in turn damages cellular components, exacerbating insulin resistance and β-cell dysfunction. Therefore, agents that possess both **antidiabetic** and **antioxidant** properties are of significant therapeutic interest.[2]

In recent years, there has been growing attention toward the use of **herbal medicines** in managing diabetes and oxidative stress. Medicinal plants offer a rich source of bioactive compounds—such as flavonoids, alkaloids, tannins, and saponins—that can modulate carbohydrate metabolism, inhibit carbohydrate-digesting enzymes, and scavenge free radicals. Unlike synthetic drugs, plant-based therapies are often perceived to have fewer side effects and are more accessible in low-resource settings.[3]

Among these medicinal plants, ***Coldenia procumbens* Linn.**, fig.1 a small prostrate herb belonging to the family Boraginaceae, holds ethnopharmacological significance in traditional Indian medicine. Traditionally used for treating inflammation, ulcers, liver disorders, and skin ailments[4] *C. procumbens* has also been cited in folk medicine for managing blood sugar levels. Despite its traditional use, scientific validation of its antidiabetic and antioxidant properties remains limited.[4]This study aims to evaluate the **in vitro antidiabetic and antioxidant activities** of the aerial parts of *Coldenia procumbens*, with a focus on α-amylase and α-glucosidase inhibition and free radical scavenging assays.[5] The results are intended to provide scientific support for its traditional use and

explore its potential as a complementary therapeutic agent in diabetes management.

II. MATERIALS AND METHODS

2.1 Plant Collection and Authentication:

In the present study, the leaves of *Coldenia procumbens* were collected from local areas of Rajampeta and Authenticated by Dr. B. Adhinarayana, Department of Botany, Government Degree College, Rajampeta, Andhra Pradesh. The authenticated leaves of *Coldenia procumbens* were air dried and subjected to size reduction to get coarse powder and it was subjected to standardization with different parameters.

2.2. Preparation of Extracts

The Leaves of *Coldenia procumbens* was air dried and reduced to coarse powder the powdered material 50gms was subjected to solvent extraction in Soxhlet apparatus with petroleum ether followed by ethanol.

2.3. Phytochemical Screening

A systemic and complete study of crude drugs should include through investigations of both primary and secondary metabolites derived as a result of plant metabolism. The different qualitative chemical test are to be performed for establishing profile of an extract for the nature of chemical compounds like alkaloids, glycosides, tannins, essential oils and other secondary metabolites using standard pro[6]

2.4. Physicochemical Parameters:

The physiochemical properties like Total ash, water insoluble ash, acid insoluble ash, loss on drying and also water soluble, alcohol soluble, extractive values are evaluated.[7]

Fig.1: *Coldenia Procumbens. L*

2.5 ATR- Spectroscopy :

Attenuated total reflection (ATR) is a sampling technique used in conjunction with infrared spectroscopy which enables samples to be examined directly in the solid or liquid state without further preparation.[8]

2.6. In Vitro Anti Diabetic Activity[9]

Method: α-Amylase Inhibition Assay

The principle involved in the amylase inhibition assay is the 3,5-dinitro salicylic acid [DNSA] is an aromatic compound that reacts with reducing sugars and others reducing molecules to form 3-amino-5-nitrosalicylicacid absorbs light strongly at 540nm it was first introduced as a method detecting reducing substances in urine and has since been widely used, for example, for quantifying carbohydrate levels in blood.it is mainly used in assay of a-amylase. However enzymatic methods are usually preferred due to DNS lack of specificity. Inhibition of a-amylase enzyme involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose after a mixed carbohydrates diet and therefore can be an important strategy in management of blood glucose. Inhibition of alpha amylase enzyme A total of (200-1000 µg/ml) of test samples and standard drug (200-1000µg/ml) were added to 500 µl of 0.20M phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5-dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle. 1gm of DNS (Dinitro Salicylic Acid)Dissolved In 2n Sodium Hydroxide 30 Gm of Potassium Sodium Tartarate Was Added And The Whole solution Was Adjusted To 100ml of Suitable Dissolving Sample.[10]

$$\% \text{ Inhibition} = \frac{Ab(control) - Ab(sample)}{Ab(control)} \times 100$$

Where, Abc =absorbance control, Abs= absorbance sample

2.7. In Vitro Anti Oxidant Activity

Method: DPPH radical scavenging activity

The free radical scavenging activity of Ethyl extract and ethanolic extract of *Coldenia procumbens* was measured in terms of hydrogen donating or radical scavenging ditty using the stable radical DPPH.Stock solution of ethanol extract of plant concentration a concentration of 1mg/ml was prepared. The following concentrations were prepared by using ethanol solvent are, 50 µg/ml, 100µg/ml 200µg/ml 400µg/ml 600µg/ml. 4mg of DPPH (0.004%) was dissolved in 100 ml of ethanol kept a side for 30 minutes in a dark place for the generation of DPPH radical and the containers were wrapped with aluminium foil to avoid any direct light exposure. (Note: DPPH is a photo sensitive, preparation of DPPH solution was done in the dark)[11]

DPPH was scavenged by an anti-oxidant through donation of hydrogen to from a stable DPPH molecule. This decrease in absorption is stochiometric according to the degree of reduction. The remaining DPPH, which corresponds inversely to the radical scavenging activity of the sample extractor antioxidant.The procedure was repeated for standard (ascorbic) acid was used as standard.[12]

Results:

Table 1: Macroscopical characteristics of leaves of *Coldenia procumbens*. Linn

Parameters (physical tests)	Observation of Leaves
Colour	Green
Odour	Aromatic
Taste	Aromatic
Texture	Smooth

Table 2: Analysis of extracts of leaves of *Coldenia procumbens. Linn*

Extract	Colour of extract	consistency
Petroleum ether	Dark Green	Solid mass
Ethanol	Yellowish green	Solid mass

Table 3: Physicochemical parameters of leaves of *coldenia procumbens.Linn*

Name of parameter	Percentage (%) w/w
total Ash value Acid insoluble ash value Water insoluble ash value	8.4% 3.2% 4.4%
Water soluble extract Alcohol soluble extract	5.20% 12.58%
Loss on drying	2.52%

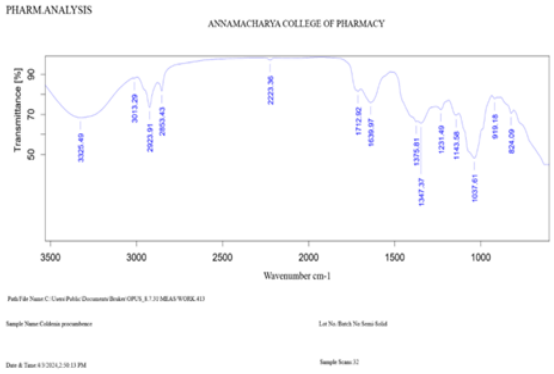

Fig.2:ATR Spectroscopy

Table 4: Interpretation of IR spectroscopy of *Coldenia procumbens*.

S.No	Wave Number	Inference
1.	3325.49	OH group
2.	3013.29	C-H group
3.	2923.91,2853.43, 2223.43	C-H aliphatic C=C, C=N, N=N
4.	1712.92, 1639.93	C=O
5.	1375.81, 1347.37	C-C
6.	1037.61, 824.09	C-H bending, (aromatic)

Table 5: Qualitative physicochemical analysis of ethanolic extract of *Coldenia procumbens.Linn*

S.No	Chemical constituents	Ethanolic extract
1.	Carbohydrates	+
2.	Proteins and amino acids	+
3.	Flavonoids	+
4.	Tannins and phenolic compounds	+
5.	Steroids	-
6.	Saponin glycosides	+
7.	Alkaloids	+
8.	Triterpenoids	+

Table 6: Effect of ethanolic extract of *Coldenia procumbens* on anti diabetic activity inhibition of α-amylase activity.

Conc.(µg/ml)	% inhibition	
	Coldenia procumbens Mean ± sem	Acarbose
200	55.69±0.08	58.86±0.032
400	59.62±0.17	63.92±0.056
600	66.96±0.08	70.63±0.185
800	72.77±0.23	76.07±0.57
1000	80.63±0.14	84.17±0.05

Table 7: Effect of ethanolic extract of *Coldenia procumbens* linn on anti oxidant activity

	Conc.	%inhibition

S. No.	(µg/ml)	*Coldenia procumbens.linn mean ± sem*	Ascorbic acid
1.	50	45.27±0.052	49.72±0.133
2.	100	51.30±0.120	57.10±0.576
3.	200	58.63±0.102	65.71±0.272
4.	400	67.30±0.058	73.4±0.120
5.	600	79.17±0.146	82.4±0.262

IV. RESULT AND DISCUSSION

Hyperglycemia[13] has been a classical risk in the development of diabetes and the complications associated with diabetes. Therefore control of blood glucose levels is critical in the early treatment of diabetes mellitus and reduction of macro and micro vascular complications.[14] One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of enteric enzymes including a-glycosidase and a-amylase present in the brush borders of intestine. Hyperglycemia is a prime characteristic of diabetes mellitus and has been a focus in the therapy for diabetes. One of the therapeutic approaches which involve decreasing hyperglycemia aims at inhibiting the enzyme a-amylase and alfa- glucosidase[15] In the present study, shade dried *coldenia procumbens (Linn)* belonging to family Boraginaceae, having medicinally important bioactive constituents is reviewed, with special emphasis on the biological activities.

The plant material was air dried and reduced to coarse powder.The powder material was subjected to solvent extraction in Soxhlet apparatus with ethanol. The soxhlation[16] was continued and until the colorless solution was obtained and the solution was concentrated in rotary evaporator and reduced pressure and yield of extract is 4.6 g. The colour of extract was reddish brown in colour with astringent taste.The ethanolic extract of *Coldenia procumbens. Linn* aerial parts was subjected for the preliminary phytochemical analysis for the presence of volatile oils, Terpenoids, flavonoids, amino acids, fats, steroids and tannins and phenolic compounds.[17]

The amylase inhibitory activity of ethanolic extract of plant was done and the results were presented in table 6. The activity was done at various concentrations of the extract and standard acarbose and the optical density was observed at 540nm. The percentage inhibition of a-amylase by the extracts of *Coldenia procumbens* was studied in a concentration range of 1000 µg/ml. Maximum *Coldenia procumbens* % inhibition of 80.63% was observed at 1000 µg/ml. acarbose a standard anti diabetic drug showed the maximum % inhibition 84.17% at the concentration of 1000 µg/ml.[18] The anti-oxidant activity[19] of ethanolic extract of plant was done and the results were presented in table. The activity was done at various concentrations (50, 100, 200, 400, 600µg/ml) of the extract and standard ascorbic acid and the optical density was observed at 517 nm. The percentage inhibition of extract of *Coldenia procumbens* was studied in a concentration rage of 1000µg/ml. Maximum *Coldenia procumbens* %inhibition of 79.17 was observed at 1000 µg/ml. ascorbic acid[19] a standard anti oxidant drug showed the maximum %inhibition 82.40% at the concentration of 1000 µg/ml.[20]

V. CONCLUSION

The present study scientifically validates the traditional use of *Coldenia procumbens Linn*. for its antidiabetic and antioxidant potential. The ethanolic extract of the aerial parts demonstrated significant inhibition of both α-amylase and α-glucosidase enzymes, with inhibitory activities comparable to the standard drug acarbose. This suggests its potential in managing postprandial hyperglycemia. Additionally, the extract exhibited strong dose-dependent free radical scavenging activity in DPPH assay, supporting its antioxidant efficacy. Phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, tannins, phenolics, and saponins, which likely contribute to these effects. Overall, *Coldenia procumbens* exhibits promising in vitro antidiabetic and antioxidant properties, warranting further research, including in vivo studies and isolation of active constituents, to explore its full therapeutic potential in the management of diabetes and oxidative stress-related conditions.

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