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PHARMOCOLOGICAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF COLDENIA PROCUMBENS LINN.

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ABSTRACT

Analysis of Phytochemical constituents and the ant diabetic & anti-oxidant activity of the medicinally important plant species *Coldenia procumbens Linn.* Collected from Thamanur village of Gingee Fort in Villupuram district of Tamil nadu, India was carried out in the present study. The fresh leaves of the above plant were used after they were dried and powdered for extraction with methanol solvent. The crude extracts were screened for the presence of phytochemical constituents and found with the presence of phenols, terpenoids and tannins in significant level and flavonoids and saponins at moderate level in the crude extracts from the leaves of *Coldenia procumbens.* The quantitative estimation of phenols and flavonoids were determined and found to be 315.63 GAE/g (Gallic acid equivalent) and 166.54 QE/g (Qurcetin equivalent) respectively. The anti-diabetic activity of methanolic crude extract of the above plant was assayed and observed as 53% (at 1000µl conc.) for Glucose uptake by Yeast cells assay, 21.96% (at conc. 125µg/ml) for inhibition of α-amylase activity test and 77.52% (at conc. 1250mg/ml) for Non-enzymatic glycosylation of Hemoglobin assay. For the antioxidant activity of the plant leaf extract, it showed a maximum of 78% by DPPH assay (at conc. 600 µg/mL), the scavenging activity reached 0.197 – 0.737% (conc. of 50 - 350µl) for Phosphomolybdenum assay and 17.08 to 65.57% (concentration from 50 - 350µl) for Nitric oxide scavenging assay. Thin Layer Chromatography (TLC) was performed on crude compound and observed the separation of 6 bioactive compounds at the RF value of 0.24, 0.38, 0.44, 0.57 and 0.64 as red color in UV light and brown color in iodine vapour with additional compound at the RF value of 0.90. From the present study, it was found that methanolic leaf extract of *C. procumbens* leaves possessed high anti-diabetic and antioxidant activity and found to have potential phytochemical compounds.

KEYWORDS: ***Coldenia procumbens, phytochemical analysis, bioactive compounds, anti-diabetic activity, Anti-oxidant activity, Thin Layer Chromatography.***

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INTRODUCTION

Plant-derived compounds have recently gained greater attention owing to their varied applications with reference to human and animals. Medicinal plants are the important resource of drugs of traditional systems of medicine, modern medicines, Nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.[1] from time immemorial the natural products have been a rich repository of remedies with diverse chemical structure and bioactivities against several health disorders including cancer. The use of plants as medicines has paved the way for isolation of active compounds, beginning with extraction of Morphine from Opium in the early 19th century. It is estimated that 122 drugs from 92 plant species have been discovered through ethno botanical leads in various parts of the world. Efficacious medicines have been discovered during innovative periods when traditional approaches were open to new ideas.[2] Diabetes mellitus (Diabetes) is one of the common metabolic disorders affecting around 2.8% of the world's population and it is anticipated to cross 5.4% by the year 2025. According to WHO, the global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 mainly in the developing countries? It is estimated that diabetes mellitus (DM) affects more than 366 million people worldwide and it is expected that this level would reach a staggering 552 million by 2030.[3] India presently has the largest number of diabetic patients in the world and has been infamously known as the 'diabetic capital' of the world.[4] Diabetes mellitus is a clinical syndrome characterized by a deficiency in insulin production or resistance to insulin action. Consequently, it leads to inappropriate hyperglycemia. Prolonged hyperglycemia can cause severe complications that probably affect every system of the body and every drug taken by the patient.[5] Diabetes mellitus is a systemic metabolic disease characterized by hyperglycemia, abnormal elevated levels of lipid and fat in blood, and hypo insulinaemia.[6] an antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as this, ascorbic acid, or polyphenols. In order to realize the health benefits from potential plant sources, it is important to measure the anti-oxidant activity using various radicals and oxidation systems.[7] the most important phyto-chemical constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds which are capable of curing various human ailments and preventing disorders. Phenolic compounds and flavonoids are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-carcinogenic activity etc.[8] Antioxidant substances, hydroxyl (oOH) group in phenolic compounds were claimed as largely responsible for their antioxidant and antimicrobial actions. Based on literature survey and available pharmaceutical potential values of plants, *Coldenia procumbens L.,* is found to be a medicinally important plant which has been selected for the present study. *C. procumbens L.* grows like an annual herb and a common weed in India.[9-10] It belongs to the family Boraginaceae.[11], which has around 150 genera and almost 2,500 species across the globe. *C. procumbens L.* is the only species of its genus which has a place both in the Hortus Bengalensi's and Moon's Catalogue of Ceylon plants. The plant is found to be effective in treating fever, piles and scorpion stings. In the traditional system of medicine, the plant was used as anti-inflammatory, anti-microbial, analgesic.[12-14]antioxidant activity[15], anti-diabetic[16], CNS depressant. The Fresh leaves of *C. procumbens L.* are powdered and applied to rheumatic swellings. Equal parts of dried powder mixed with seeds of fenugreek causing suppurations of boils were also proved to be efficient.[17] Therefore; the present study is focused on the effect of ant diabetic and antioxidant activity of *C. procumbens* Linn. by using methanolic leaf extract. Plant samples were collected from Thamanur village of Gingee Fort in villupuram district of Tamil Nadu, India for the analysis of phytochemical potential.

MATERIALS AND METHODS

Collection of leaf samples and Preparation

The plant species *Coldenia procumbens Linn*. was identified by using The Flora of the Presidency of Madras by Gamble in our laboratory and further confirmation and authentication was done by Dr.S.Narashimman, Associate Professor of Botany (Taxonomist), Madras Christian College, Chennai, Tamil Nadu. The dried specimen in the form of herbarium is preserved in our Botany Laboratory for further reference. The leaves were air dried and powdered in to fine material. The powdered final material is labeled and stored in a air tight container in our laboratory. Fresh leaves of *Coldenia procumbens* Linn. Were collected from Thamanur village of Gingee Fort in villupuram district of Tamil Nadu, India. The leaves were carefully washed with tap water followed by rinsing in distilled water and air-dried at room temperature for few hours. Then leaves were separated and taken to a separate clean place and dried at room temperature for one week. Then they were ground into fine powder and sieved through fine mesh, finally stored in a cool and dry place in a clean air-tight container.

Figure 1
Morphology of Coldenia procumbens Linn.

Extraction of Sample
Extraction of leaf powder with chloroform, ethyl acetate and methanol was performed by direct method of extraction after the method of Eloff, 1998.[18] Finely ground plant material was extracted with chloroform, ethyl acetate and methanol in the ratio of 1:10 in conical flask in shaking condition for overnight. The extract was filtered through the Whitman No. 1 filter paper in a separate container. The above process was repeated 3 times with the same plant material but using fresh solvent. The solvent was removed by placing the extracts in distillation unit in the respective temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/ml solutions ready for further analysis.

Qualitative Phytochemical Analysis
Test for Phenolic compounds (Ferric chloride test) [19]
The methanolic plant leaf extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenol.

Test for Glycosides -Borntrager's test[20]
About 50mg of methanolic plant leaf extract was hydrolysed with 5ml of concentrated hydrochloric acid for two hours on a water bath and filtered. To 2ml of filtrate hydrosylate, 3ml of chloroform was added and shaken well. Then the chloroform layer was separated and 10% ammonia solution was added to it. Appearance of a pink, red or violet color in the ammoniacal (lower) phase indicated the presence of glycosides.

Test for Terpenoides - Salkowi test[21]
About 0.5g of methanolic leaf extract was added in 2ml of chloroform and concentrated sulphuricacid (3ml) and was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Flavonoids - Sodium hydroxide test
About 0.5g of methanolic leaf extract was dissolved in 5ml of distilled water and filtered. To 2ml of filtrate, few quantity of each portion was dissolved in water and filtered. To this, 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.[22]

Test for Tannins - Neutral Ferric Chloride[22]
About 0.5g of methanolic leaf extract was boiled in 10ml of water in test tube and then filtered. A few drops of 1% ferric chloride was added and the appearance of blue-green, green or brownish green precipitate indicates the presence of tannins.

Test for Reducing Sugars - Fehling's test[23]
The methanolic leaf extract (100mg) was dissolved in 5ml of water and filtered. About 1 ml of filtrate was boiled in water bath with 1ml each of Fehling's solution I and II.A red precipitate indicates the presence of reducing sugars.

Test for Saponin - Foam test[24]
The methanolic leaf extract(50mg) was diluted with 5ml of distilled water .The suspension was shaken in a graduated cylinder for 15 min. Appearance of 2cm layer of thick foam indicates the presence of saponins.

Test for Protiens - Biuret test[25]
The methanolic leaf extract (100mg) was dissolved in 10ml of distilled water and filtered through Whitman No.1 filter paper and the filtrate was collected. To 2ml of filtrate, one drop 2% of copper sulphatesolutionand1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets (1pellet). Appearance of Pink color in the ethanolic layer indicates the presence of proteins.

Quantitative Phytochemical Analysis
Determination of Flavanoids by Aluminium chloride test[26]
To 1ml of varying concentrations of leaf extract, 3 ml of methanol, 0.2ml of 1 M potassium acetate, 0.2ml of10% aluminium chloride and 5.6ml of distilled water were added and left at room temperature for 30 minutes. Absorbance of the mixture was read at415 nm using UV–VIS spectrophotometer. Calibration curve was

prepared using Quercetin as standard and estimated the value of flavonoids content.

Determination of Phenolic compounds by Folin Ciocaltaeu's method[2]

The total phenol content of the extract was measured at 765 nm by Folin-Ciocalteu reagent.[2] The dilute methanolic leaf extract (0.5 ml of 1:10 g ml-1) and or gallic acid (standard phenolic compound) was mixed with 5ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 4ml of aqueous sodium carbonate (1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared by using 0, 50, 100, 150, 200, 250 mg/lsolutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent.

Ant -diabetic Assay

Glucose uptake by Yeast cells

Commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1ml of glucose solution (5, 10 and 25 mg) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant by using spectrophotometric method. Metronidazole was taken as standard drug.[27] the percentage increase in glucose uptake by yeast cells was calculated using the following formula

Increase in glucose uptake (%) = {*Abs sample – Abs control\Abs sample*}*100

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

Inhibition of α-amylase activity

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of0.20 mm 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 and cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. [28]

% inhibition of α-amylase activity = (Abs sample X Abs control/Abs sample)*100

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Non-enzymatic glycosylation of Hemoglobin

Glucose (2%), hemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M at pH 7.4. About 1 ml each of above solution was mixed in a test tube. The methanolic extract was weighed and dissolved in DMSO to obtain stock solution and then 1-5 µg/ml solutions were prepared. Then 1 ml of each concentration was added to above mixture. The Mixture was incubated in the dark at room temperature for 72hrs. The degree of glycosylation of hemoglobin was measured by spectrophotometer at 520nm.[27] Metformin was used as a standard drug for assay and % inhibition was calculated using the formula

% inhibition = (Abs sample X Abs control/Abs sample)*100

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample

Antioxidant Assay

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The Radical Scavenging Activity (RSA) of different extracts was determined by using DPPH assay according to Chang *et al* (2008) with minor modification.[29] The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2.96ml of 0.1 ml Methanolic DPPH solution mixed with 40 µl of *C. procumbens L.* (20 to 200µg/ml) respectively and vortexes thoroughly. The setup was left at dark in room temperature and the absorption was measured calorimetrically at 517nm after 20 minutes. Alpha tocopherol was used as standard for reference. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

% of DPPH Radical Scavenging Activity = (Abs control - Abs sample/Abs control) * 100

Abs control is the absorbance of DPPH radical + ethanol; Abs. sample is the absorbance of DPPH radical + plant extract. Measurements were performed in triplicates. Absorbance values were corrected for radicals decay using blank solutions.

Phosphomolymbdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto et al (1999).[30] An aliquot of 100µl of sample solution was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium moly date) in a 4ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

Reducing power assay

The reducing power of the extracts was evaluated according to Oyaizu, 1986.[31] Different amounts of aqueous extracts were perched in aqueous solvent and diverse with 2.5 ml of 0.2M phosphate buffer (pH 6.6), and 2.5 ml of 1% K3Fe (CN)6. This mixture was incubated at 50° C for 20 min. To this mixture, 2.5 ml of 10% TCA was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was assorted with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicates increased reducing power. All the tests were performed in triplicates and the results were pooled and expressed as mean values.

Nitric oxide scavenging assay[32]

Methanolic leaf extract was dissolved in distilled water for this quantification. Sodium Nitro preside (5mM) with standard phosphate buffer saline (0.025m, pH 7.4) was incubated with different concentration (100-400µg/ml) of methanol extract and tubes were incubated at 29°C for 3 hours. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagents. The absorbance of the colour developed during diazotization reaction was observed at 550nm on spectrophotometer. Ascorbic acid was used as a standard for comparison with plant extract sample and calculated the % inhibition.

% of inhibition = (O.D. of control – O.D. of sample/O.D. of control) * 100

Separation of bio-compounds by Thin Layer Chromatography (TLC)

Thin layer chromatography technique for separation of active plant compounds from methanolic leaf extract was achieved after the method of Chang*e al.,* 2013.[33] Silica gel TLC plate obtained from Merck laboratories about 1.5cm wide and 5cm long was used. Marking was done on the TLC plate using a pencil about 1cm above the bottom edge. A small quantity (few microliters) sample solution was spotted on the silica gel plate. The sample is allowed to completely evaporate for better separation of compounds achieved during chromatogram. A small amount of an appropriate solvent (eluent) poured into a glass container called TLC chamber (separation chamber) to a depth of less than 1 centimeter. The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber. Then the chromatogram chamber is closed with a lid and is left for 10 minutes for saturation. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). When the solvent front reaches top of the glass plate less than 1 cm to the edge, the TLC plate was removed and air dried. Than the sample spots appeared in the UV lamp were noted and marked with pencil for calculation of RF values. Further, the dried plate is placed in a chamber containing a few crystals of iodine and the iodine vapors in the chamber oxidizes the substances with e various spots, making them visible to the eye. Once the spots are visible, they were e outlined with a pencil before the iodine coloration fades. The Rf values of sample spots were calculated with the following formula.

$$\text{Rf value} = \frac{\text{Distance traversed by the compound}}{\text{Distance traversed by solvent}}$$

STATISTICAL ANALYSIS

All the experiments were performed in triplicate to avoid experimental error. The results of the present study were analyzed statically and expressed as mean (n=3) +/- Standard Deviation (SD).

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

The screening tests for phytochemical constituents revealed the presence of phenols, terpenoids and tannins in moderate to high level and Glucosides, flavonoids and saponins at low evel in crude extracts from the leaves of *C. procumbens L*. Proteins are found to be absent in leaf extract sample. The details are presented in Table 1.

Table 1

Qualitative and quantitative phytochemical analysis of C. procumbent Linn.

S.no	Phytochemicals	Qualitative pattern	Quantitative pattern
1	Phenolic compound	Moderate	315.63 GAE/g
2	Glycosides	Low	--
3	Terpenoids	High	--
4	Flavonoids	Low	166.54 QE/g
5	Tannins	Moderate	--
6	Reducing sugars	Moderate	--
7	Saponins	Low	--
8	Proteins	Absent	--

Quantitative Phytochemical Screening

Detection of flavonoids

Quantitative analysis of the methanolic extract of plant leaves for flavonoid content shows significant amount when compared to the standard Quercetin. In the extract, the quantity of flavonoid was found to be 166.54 QE/g, in *C. procumbens* (Table 1).

Detection of Phenolic compound

Quantitative analysis of the methanolic extract of plant leaves for phenolic compounds shows in significant level compared to the standard Gallic acid. The amount of phenolic compound present was found to be 315.63 GAE/g, in *C.procumbens,* leaf samples (Table 1). Aleemuddin et al., (2011)[34] analyzed the psychopharmacological properties in the entire plant of *C. procumbens* and concluded that alkaloids and tannins are higher in alcoholic extracts than in water extract, reducing sugars and phenols are higher in water extract than in alcoholic extract. Non-reducing sugars and steroids are equally present in both the extract. Senthamarai et. al., (2002)[12] found saponins, and fixed oils and fat are present only in water and alcoholic extract respectively. Ganesan et al., (2014)[15]analysed the flavonoids and the total Phenolic content in methanolic extract of *C. procumbens* leaves and reported 100µg/ml concentration contains 12.0943µg BHT equivalent of total phenol. Apart from the *C. procumbens,* many plants were analyzed for qualitative phytochemical screening and the medicinal plants and found mainly with the presence of phenols and flavanoids. Phenol and Saponin were identified in methonolic extract of *Moringa concanensis* leaves, flower and seeds.[35]

Anti-diabetic assay

Inhibition of α-amylase activity

For the assay of ant diabetic activity by α-amylase test, the methanolic extract of *C. procumbens L.* showed significant inhibitory activity with inhibition percentage of 1.91 – 21.96% (concentration of 25 - 125µg/ml). The results are indicated in table 2 and graph 1.

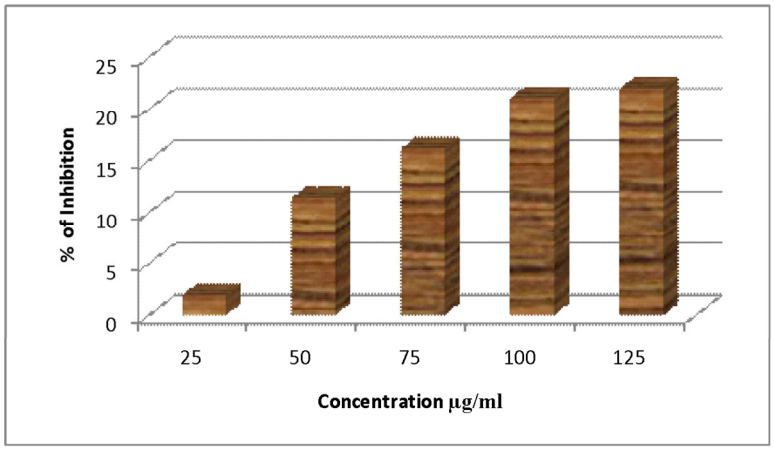
Table 2

Glucose assay alpha-amylase of C.procumbensLExtract

Sl.No.	Concentration (mg/ml)	% of inhibition
	Control	
1	25	1.91 ± 0.13
2	50	11.37 ± 0.79
3	75	16.13 ± 1.12
4	100	21.03 ± 1.47
5	125	21.96 ± 1.53

Mean values are +/- SD (n=3)

P values are less than 0.01 compared with control



Graph 1

Glucose Assay Alpha-Amylase– C. procumbens L.

Non Enzymatic Glycosylation of Hemoglobin

The enzyme inhibitory activity of methanolic extract of *C. procumbens,* on glycosylation of haemoglobin was found and compared with the standard drug acarbose. Results showed that the extracts of *C. procumbens* have inhibitory activity from 7.14% to 77.52 in the concentration of 250 mg/ml to 1250 mg/ml. The results are presented in Table 4;

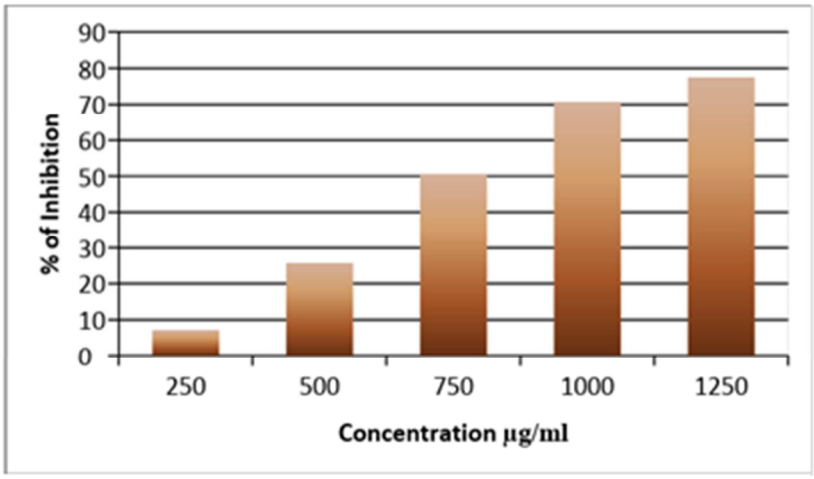
Table 3

Non-enzymatic Glycosylation of Haemoglobin of C. procumbens L

S.No	Concentration (mg/ml)	% of inhibition
1	250	7.14 ± 0.49
2	500	25.71 ± 1.79

3	750	50.63 ± 3.54
4	1000	70.78 0± 4.95
5	1250	77.52 ± 4.98

Mean values are +/- SD (n=3)
P values are less than 0.01 compared with control



Graph 2
Non–enzymatic glycosylation of Haemoglobin of C. procumbens L

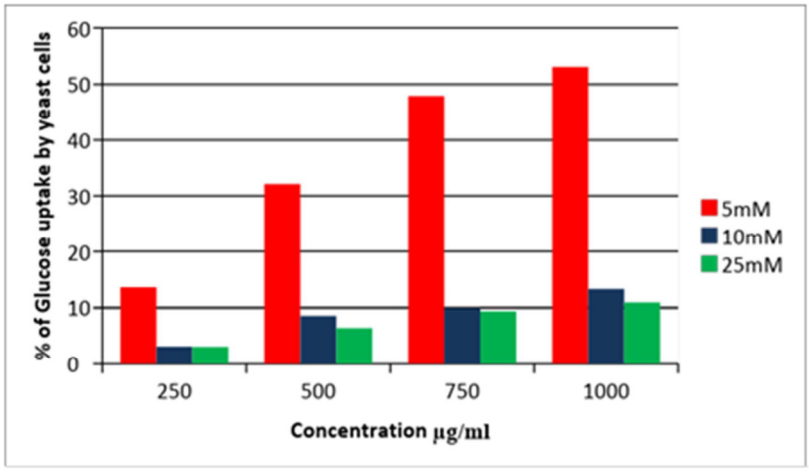
Glucose Uptake by Yeast Cells

Transport of glucose across yeast cell membrane was studied in an *in vitro* system comprising of yeast cells suspended in glucose solution of varying concentration in the presence of the plant leaf extracts. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The inhibitory activity of methanolic extract of *C. procumbents L* was found and compared with the standard drug acarbose. Results showed that the inhibitory activity is higher in increased concentration from 250 ul to 1000 ul which is compared with standard drug. The details of results were presented in Table 4; Graph 3.

Table 4
Glucose uptake by yeast cells – C. procumbens L

S.No.	Concentration (µl)	Percentage of Glucose uptake by yeast cells		
		5mM	10mM	25mM
1	250	13.63 ± 0.95	3.12 ± 0.21	3.07 ± 0.21
2	500	32.14 ± 2.24	8.54 ± 0.59	6.40 ± 0.44
3	750	47.94 ± 3.35	9.95 ± 0.69	9.42 ± 0.65
4	1000	53.08 ± 3.71	13.38 ± 0.93	10.98 ± 0.76

Mean values are +/- SD (n=3)
P values are less than 0.01 compared with control



Graph 3
Glucose uptake by yeast cells – C. procumbens L

Regarding the diabetic activity of *C. procumbens L,* in 2007, Patel ET. al., [16] evaluated the ant diabetic activity with alloxan-induced diabetes in rat. Treatment with *C. procumbens L* significantly ($p < 0.05$) reduced the blood glucose level from 394.17 ± 10.52 (mg/dl) to 152.83 ± 2.15 (mg/dl) in rats when compared with diabetic control group of rats. Serum triglyceride levels decreased significantly ($p < 0.05$) from 152.33 ± 2.75 (mg/dl) to 109.17 ± 1.74 (mg/dl) and reduced the serum cholesterol level from 59.83 ± 1.01 (mg/dl) to 44.33 ± 1.96 (mg/dl). The analysis of data indicates that the test drug has good hypoglycemic effect in diabetic rats. Apart from the *C. procumbens,* Abhijit Sonawan et.al. (2014)[36] studied in-vitro alpha amylase activity of various solvent extract of *Tridax procumbens* and found α-amylase enzymes activity of methanolic plant exracts exhibit high value of antidiuretic activity in comparison to positive control (Acarbose).

Anti-oxidant assay

DPPH assay: (2, 2-diphenyl-1-picrylhydrazyl)

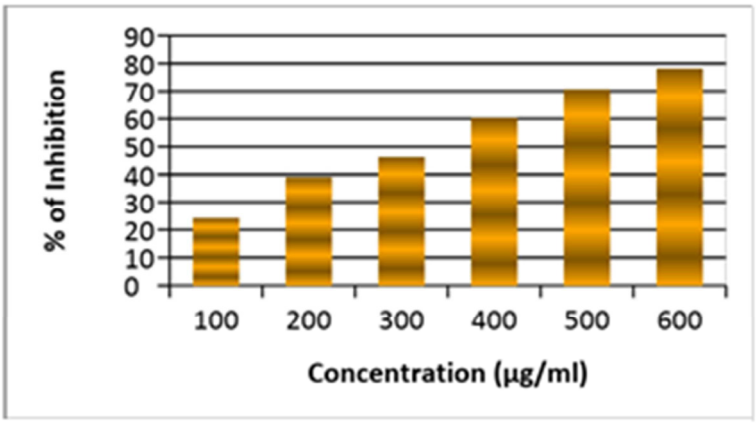
From the dose dependent response curve of DPPH radical scavenging activity of methanolic leaf extract of *C. procumbens L.* showed higher inhibition activity as 78%in the concentration of 600 µg/ml. But 50% scavenging activity was calculated as 325 µg/ml concentration. The details of concentration values and the scavenging activity were shown in the Table 5, Graph 4.

Table 5

DPPH radical scavenging activity of C. procumbens L

S.No	Concentration (µg/ml)	% of Inhibition
1	Control	-
2	100	24.17 ± 1.69
3	200	39.10 ± 2.73
4	300	46.26 ± 3.23
5	400	60.29 ± 4.22
6	500	70.74 ± 4.95
7	600	78.20 ± 5.47

Mean values are +/- SD (n=3)
P values are less than 0.01 compared with control



Graph 4

DPPH Radical scavenging activity of C. procumbens L

Percentage of inhibition = control OD – Sample OD/control OD * 100

Phosphomolybdenum assay

The Phosphomolybdenum reduction assay was based on the reduction of Mo (VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature. The Phosphomolybdenum reduction assay values increases with increase in concentration of methanol extract of leaves of *C. procumbens.* The results are shown in Table 6, Graph 5.

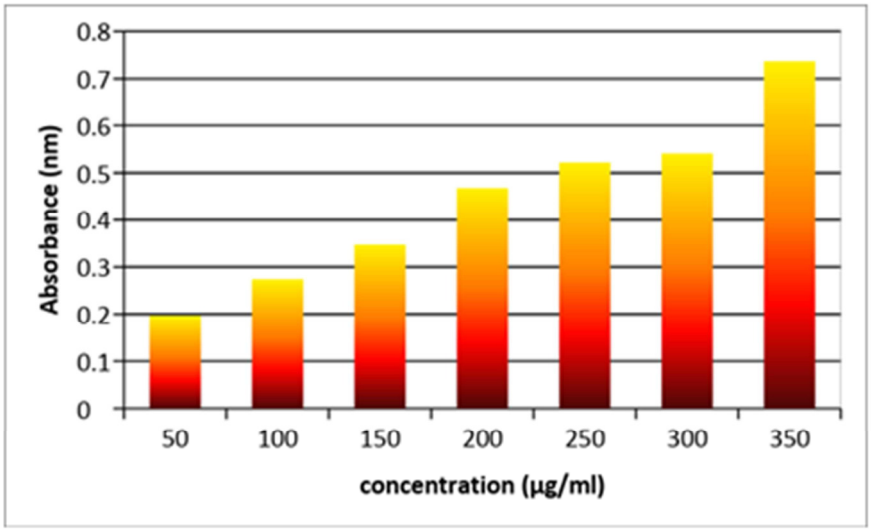
Table 6

Phosphomolybdenum assay –C. procumbens L.

S.No	Concentration(µg/ml)	Absorbance @ 695 nm
1	Control	-
2	50	0.197 ± 0.01
3	100	0.274 ± 0.01
4	150	0.347 ± 0.02
5	200	0.468 ± 0.03
6	250	0.522 ± 0.03

7	300	0.541 ± 0.03
8	350	0.737 ± 0.05

Mean values are +/- SD (n=3)
P values aree less than 0.01 compared with control



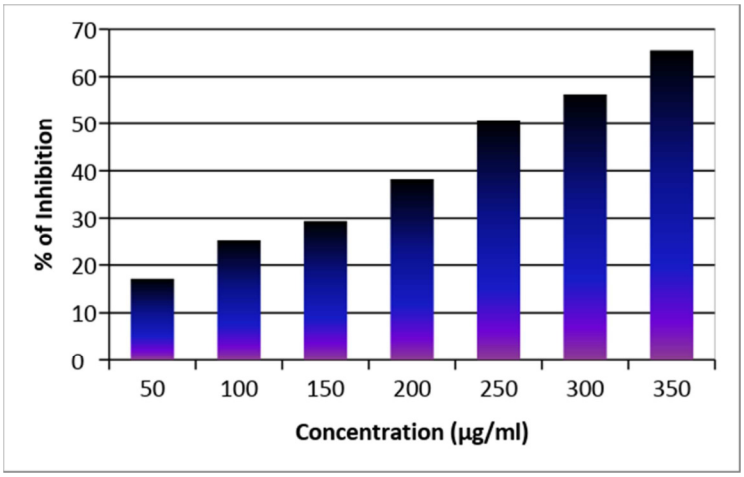
Graph 5
Phosphomolybdenum assay C. procumbens L

Nitric oxide scavenging assay
Maximum inhibition of NO was observed in the plant leaf extracts of highest concentration as 350µg/ml for *C. procumbens L.* The assay value indicates that at the maximum concentration, inhibition was found to be 65.57% in comparison with ascorbic acid, which serves as the standard. The values are presented in Table 7 and Graph 6.

Table 7
Nitric oxide scavenging activity of C. procumbens L

S.No	Concentration (µg/ml)	% of Inhibition
1	Control	0
2	50	17.08 ± 1.19
3	100	25.12 ± 1.75
4	150	29.39 ± 2.05
5	200	38.19 ± 2.67
6	250	50.79 ± 3.55
7	300	56.28 ± 3.93
8	350	65.57 ± 4.58

Mean values are +/- SD (n=3)
P values are less than 0.01 compared with control



Graph 6
Nitric oxide scavenging activity of Coldenia procumbens

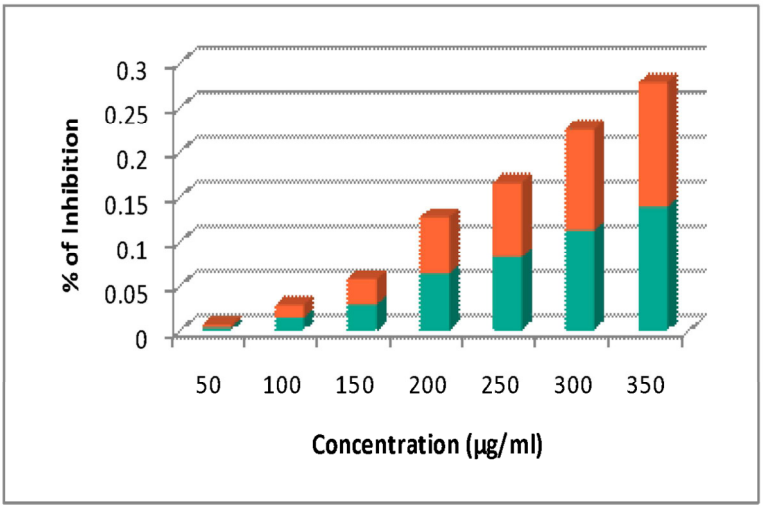
Fe^{3+} reducing power assay

The antioxidant activities of *C. procumbens* L leaf extract evaluated using reducing power assay were depicted in Table 8, and Graph 7. The results of antioxidant activity by Fe3+ reducing power assay in the methanolic extracts of *C. procumbens L* showed increased ferric reducing power with the increased concentration as standard antioxidants.

Table 8
Fe^{3+} Reducing power assay – C. procumbens L

S.No	Concentration (µg/ml)	Absorbance @ 700nm
1	**Control**	-
2	50	0.006 ± 0.001
3	100	0.028 ± 0.001
4	150	0.057 ± 0.002
5	200	0.127 ± 0.006
6	250	0.165 ± 0.008
7	300	0.225 ± 0.011
8	350	0.278 ± 0.013

Mean values are +/- SD (n=3)
P values are less than 0.01 compared with control



Graph 7
Fe^{3+} Reducing power assay – C. procumbens L

Ganesan *et al.,* (2014), [15] evaluated the antioxidant potential of whole plant alcoholic extract of *Coldenia procumbens Linn.* By using DPPH Radical-Scavenging activity, ABTS radical cation scavenging activity, Ferric-reducing power (FRAP) assay, In vitro anti-lipid peroxidation assay (using TBARS) and Superoxide Scavenging Assay and concluded that the alcoholic extract showed significant antioxidant potential was observed in all these assays. Further they found the Total phenolic content of the extract in 1000 µg/ml was equivalent to 200 µg/ml of gallic acid and concluded that the satisfactory level of antioxidant activity of whole plant alcoholic extract from Coldenia *procumbens* Linn.. The present study shows 50% inhibition on antioxidant activity at 325 (µg/ml) by using DPPH assay and 250 (µg/ml) by using nitric oxide assay, which concludes the methanolic extract of *C. procumbens L.* has a good antioxidant activity and its importance is once again highlighted for reducing the damaging oxidative stresses occurring normally in human body.

Separation of Compounds by Thin Layer Chromatography

The solvent system used for chromatogram in the ratio of 1:9 (Ethyl Acetate: Toluene v/v), shows better separation of compounds and it is most distinct and clear. This solvent system of chromatogram was chosen for further study on separation and identification of anti-diabetic compounds present in the leaves of *Coldenia procumbnens*, under the influence of UV, 5 compounds were recognized commonly and the RF value (retention factor) were calculated. Iodine was used as a reaction reagent for the compounds present in the chromatogram and 6 compounds were recognized. The RF values of compounds from *C. procumbnens L.* Were calculated and found as 0.24, 0.38, 0.44, 0.57 and 0.64 as red colour in UV light and brown colour in iodine vapour with additional compound at the RF value of 0.90 in iodine vapour. The results of RF values of different spots identified as the compounds are shown in Table 9 and Figure 2.

Table 10
Details of Compounds separated from C.procumbens L in TLC

S.No.	Ratio	Rf value UV long/UV short	Iodine
1	1:9	0.24	0.24
2	1:9	0.38	0.38
3	1:9	0.44	0.44
4	1:9	0.57	0.57
5	1:9	0.64	0.64
6	1:9	-	0.90

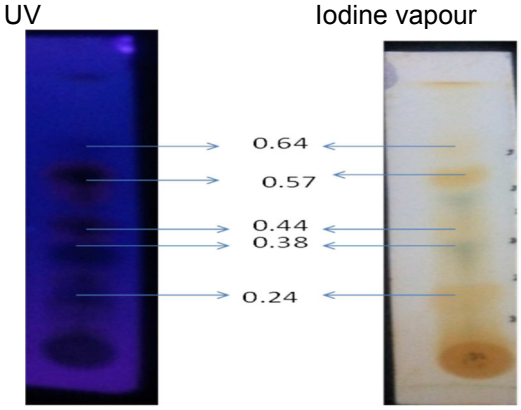


Figure 2
Sample spots separated from C. procumbens L by TLC

This study presents a review on the *in vitro* anti-diabetic effect of *C. procumbens L,* in the assessment of diabetes which provides promising results for the utilization of the extracts of the plant as a formulation for the drug to treat diabetes. The main aim of this study involves the identification of the active compound in the plant which can play an important role in the hypoglycaemic actions for the treatment of diabetes. This work highlights the use of chromatogram to identify and purify the desired compound. The basis of the work revolves around the use on desired solvents to extract the active compounds and further investigated by the *in vitro* anti-diabetic assay which useful in determination of mechanism of action of the anti-diabetic and antioxidant effect of the plant based compounds. Phytochemical screening of different active compounds such as alkaloid, flavonoid, phenolic compounds which were investigated for several in vitro assays and showed higher inhibitory activity than the other compounds present in the plant and its concentration was evaluated which provides a basis for the plant extract to be used in the treatment of diabetes and tumors. The compounds appearing in both UV light and iodine vapor with same RF values indicates that the same category of compounds is presumed to be responsible for anti-diabetic and antioxidant activity of plants *C. procumbnens L.*

CONCLUSION

Among the various solvents used for extraction of plant leaves, methanol is suitable solvent system for extraction of leaves of *C. procumbens L* which exhibited higher ant-diabetic activity and this study offers the use of the plant extract in the management and treatment of diabetes. Likewise, the methanol extract of the plant leaves possess higher antioxidant activity when compare to other solvent extract. However, the study also offers the use of the plant extract in the management and treatment of tumor and anti-proliferative activity. Further, other active compounds from the plant must be investigated and its potency must be evaluated for its various pharmacological activities. The clinical trials should also be conducted before the plant extract is taken for further pharmacological evaluation. Overall, the above plant species *Coldenia procumbens L.* can be a potential medicinal plant which will be useful in isolation, identification and development of new drug for curing various human and animal disorders.

AUTHOR CONTRIBUTION STATEMENT

Jayaraman P – Supervised and guided the overall research work and involved in the final shaping of the research article for publication. Rajesh V – Senior Research Scholar who conceived the idea and helped in the experimentation of research work and compiling of the research data and findings. Logambal R – A Junior research scholar involved in the conduction of experiments, observation and preparation of the article.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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