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Research Article

ISOLATION ANTI-DIABETIC AND ANTIOXIDANT EVALUATION OF AQUEOUS EXTRACT OF CANSJERA RHEEDII LEAVES

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ABSTRACT

The plant *Cansjera rheedii* belonging to the family Opiliaceae was selected and various studies such as phytochemical and pharmacological activities were carried out.Plant leaves were collected and was shade dried for 15 days, until it was dry enough for extraction. It was then powdered in a grinder and extracted with solvents such as hexane, ethanol, and water.The extracts were subjected to various preliminary phytochemical tests and the results indicated the presence of flavanoids, in the aqueous and alcoholic extract. The compounds were identified by TLC and isolated by column chromatography.The spectral studies were carried out for the aqueous extract of *Cansjera rheedii* leaves and were subjected for purity testing. The purified fractions were subjected to IR (Infra red spectroscopy), ^{1}H NMR(Nuclear Magnetic resonance spectroscopy), MASS Spectroscopy studies for the confirmation of the functional groups, number of protons and molecular weight.The results obtained from the chemical tests, IR, ^{1}HNMR, MASS spectroscopy gave the conclusion that Quercetin-3-O-β-rutinoside was present in the extract.The anti-diabetic activity of the aqueous extract of *Cansjera rheedii* leaves were compared with that of standard drugs. The aqueous and ethanolic extract of *Cansjera rheedii* leaves produced significant anti diabetic activity (at doses 200mg/kg and 400mg/kg) in dose dependent manner. The in vitro antioxidant activity was evaluated using DPPH scavenging method at 25, 50, 75,100,250 µg/ml aqueous and ethanolic extracts of *Cansjera rheedii.* At 25µg/ml ethanolic extract of *Cansjera rheedii* was found to have better ($p<0.01$) DPPH scavenging activity when compared to aqueous extracts of *Cansjera rheedii.*

Keywords: *Cansjera rheedii*, antidiabetic activity, antioxidant activity, ^{1}HNMR, Mass spectroscopy.

INTRODUCTION

Diabetes is a disorder which is quite common in the modern world due to changed life style and food habits. It is characterized by hyperglycemia. Synthetic drugs like insulin, oral hypoglycemic are available for treatment but accompanied by side effects a lot of herbs which can be used for treating diabetes have been indicated in Ayurveda.

Oxidative stress represents a shift towards the pro-oxidant/antioxidant balance that can occur as a result of an increase in oxidative metabolism. Biochemical defects related to diabetic complications may arise from overproduction of reactive oxygen species/nitrogen species. ROS (reactive oxygen species) induced elevation in glucose can result in damage of DNA, proteins, lipids, decrease insulin mRNA [1,2] .

Cansjera rheedii (Family: Opiliaceae) is a climbing shrub commonly known as kalimanakeerai in tamil is found in India, China, Honkong and Australia.

Cansjera rheedii has been reported to have anthelmintic, hepatoprotective, antipyretic, diuretic activities [1,2].

Future Scope

Further studies can be done on the constituents responsible for anti-diabetic activity and the mechanism by which Quercetin-3-O-β-rutinoside exhibits antidiabetic property, antioxidant property.

MATERIAL AND METHODS

The study was carried into various sections as follows.

Collection and authentication of plant material

Leaves of *Cansjera rheedii* belonging to the family opiliaceae were collected from parts of Malappuram District of Kerala. It was identified and confirmed by botanist.

Animal approval

The study was conducted after obtaining the approval from Institutional Animal Ethics Committee (IAEC), and the experimental procedure were in accordance to the guidelines of IAEC (No:688/02/c/CPCSEA).

Preparation of Extract [3, 4, 5, 6, 7]

The powdered leaves of *Cansjera rheedii* were defatted with petroleum ether extracted with water successively by hot water reflux and continuous hot percolation method. The temperature and the duration of the extraction procedure were determined using investigational studies. An aliquot portion of the collected sample was transferred to a high grade round bottom flask and continuously refluxed for a calculated time period of three hours at a pre-determined extraction temperature of 60-70°C. Subsequently, alcohol precipitation was followed (using various concentration) and the precipitate obtained was redissolved in water. The extracts were concentrated under reduced pressure and refrigerated.

Preliminary Phytochemical Studies

Preliminary phytochemical tests were performed for chemical constituents such as alkaloids, carbohydrates, glycosides, steroids, tannins, proteins and aminoacids, fixed oils and fats, flavonoids and saponins and it was found that flavonoids were present.

CHARACTERIZATION

The isolated fraction was characterized using Column chromatography, I.R, ^{1}HNMR and Mass spectroscopy.

Experimental Animals

Male albino rats of Wistar strain and weighing about 100-200 gm were used for the study. The animals were got from Nanda College of pharmacy and research institute, Erode, and were approved by Ethical committee (NCP/IAEC/PG-2010/20).

The animals were maintained at room temperature 24 ±2^{0}C. The animals were housed in large spacious hygienic cages during the course of the experimental period.

The animals were fed with rat pellets feed supplied by M/s Hindustan Lever Limited, Bangalore, India and filtered water *ad libitum*. Animals described as fasted were deprived of food for ≥16 hr but allowed free access to water. The place where the experiments were conducted was kept very hygienic by cleaning with antiseptic solution, as the diabetic animals are susceptible to infections.

Acute Oral Toxicity Studies

The acute oral toxicity study was done according to the OECD guidelines 425.

A starting dose of 1000mg/kg body weight of aqueous extracts of *Cansjera rheedii* were administered orally to 5 male albino Wistar rats, observed for fourteen days for behavioral changes and mortality [8].

Antidiabetic Activity [9,10, 11]

Different groups of rats were used to study the effect of aqueous extract of *Cansjera rheedii*. The rats were divided into five groups each consisting of six rats:

Group 1: The rats received 2 ml Carboxy Methyl Cellulose (CMC). These animals serve as normal controls.

Group 2: Received a single dose of (150mg/kg body weight), Alloxan monohydrate in CMC through intraperitoneal route and served as negative control.

Group 3: Received the aqueous leaf extract 200mg/kg for 21days and served as test 1.

Group 4: Received the leaf extract 400mg/kg for 21 days and served as test 2.

Group 5: Received Glibenclamide 5mg/kg for 21days and served as positive control.

Induction of Diabetes

Animals were allowed to fast 24 hr and were injected with freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg i.p.). After a week rats with marked hyperglycemia (fasting blood glucose>200mg/dl) were used for the study [18] .

Determination of blood glucose

Blood was collected for the measurement of blood glucose from the tail vein at 0, 1, 2 and 3 hr after feeding the plant extracts. The blood glucose levels were determined by using one touch glucometer.

Biological estimation

After the experimental regimen, the blood was collected through the retro-orbital puncture of eye of animals under mild diethyl ether anesthesia in Eppendorff's tube (1ml) containing 50µl of anticoagulant (10% trisodium citrate) and the serum was separated by centrifuging at 6000rpm for 15min. The biochemical parameters, cholesterol, triglycerides, total protein, SALP, SGOT and SGPT are determined by using the commercial kit available (Ecoline, manufactured by Merck Specialities private limited, Ambernath.)

Statistical Analysis

The collected data were subjected to appropriate statistical test including one way ANOVA, followed by an appropriate Dunnett's t-test, p value of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using graph pad prism software.

RESULTS AND DISCUSSION

Phytochemical Studies and Characterization

The leaf extract of *Cansjera rheedii* were evaluated phytochemically and the results are represented in table 1 and 2.

Table 1: Percentage yield of Aqueous extract *Cansjera rheedii* leaves.

Leaf Extract	%Yield(w/w)
Aqueous extract	8.5 %

Table 2: Chemical constituents present in the aqueous extract of *Cansjera rheedii* leaves Present (+)Absent (-)

Phytoconstituents	Aqueous extract
Alkaloids	-
Carbohydrates	-
Fixed oils and fats	-
Flavonoids	+
Gums and mucilage	-
Glycosides	-
Proteins and amino acids	-
Saponins	-
Steroids	-
Terpenoids	-

The isolated fraction was characterized using Column chromatography, I.R, ^{1}HNMR and Mass spectroscopy. The results indicated the presence of Quercetin-3-O-β-D-rutinoside like flavanoid which is responsible for the hypoglycemic effect. Further studies are required to elucidate the structure of the Quercetin-3-O-β-D-rutinoside.

Table 3: Data for column chromatography

Fraction	Solvent	Ratio	Nature of residue	Analysis by TLC
1-5	Chloroform: ethanol	100	No residue	No spot
16-25	Benzene: Chloroform	50:50	No residue	No spot
26-35	Benzene: Chloroform	75:25	No residue	No spot
47-60	Chloroform: Water	85:15	Yellow residue	Single spot
60-72	Chloroform: Water	50:50	Brownish yellow	Two distinct spot

Table 4: IR Interpretation of the isolated fraction

Functional groups	Wave number (cm^{-1})
Aliphatic -OH stretching	3650.80
Aliphatic C-H stretching	2975.96
Aromatic C-H stretching	3058.22
Ether C-O-C stretching	1250.12

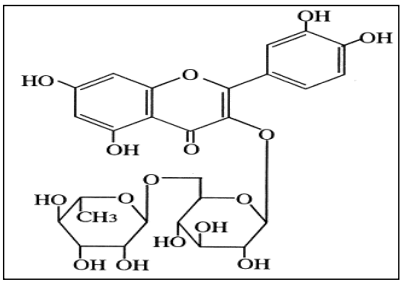
^{1}HNMR (500MHZ) DMSO(d_6) Interpretation of the isolated fraction:

δ 12.12 (s,1H,5-OH), δ10.56(s,1H,OH-7), δ 9.38 (s,1H,OH-4'), δ 9.11 (s,1H,OH-3'), δ 7.52 (d,1H,H-2'), δ 7.49 (d,1H,H-6'), δ 6.82 (d,1H,H-5') ,δ 6.37(d,1H,H-8) δ 6.16 (d,1H,H-6) of aglycone, δ5.40(d,1H,H-1''), δ 3.30 (d,1H,H-2''), δ 3.22 (d,1H,H-5'').

MASS Interpretation of the isolated fraction:

Mass: m/z 633.23(M+1)

The structure of the fraction was elucidated as below as Quercetin-3-O-β-D-rutinoside



Antidiabetic Activity

Table: 5: Effect of aqueous leaf extract of *Cansjera rheedii* on fasting blood glucose level in Alloxan induced diabetic rats.

GROUPS	1st Day	1st week	2nd week	3rd Week
CMC (0.5 %)	88.5±3.81	98.33±6.41	105.33±5.28	101.33±2.82
Diabetic control (150mg/kg Alloxan)	135±7.41	255±6.85	274.16±6.11	289±6.53
Test-I(Aq. extract -200mg/kg)	105.83±6.93**	161.83±8.75**	158±8.87**	135.5±3.21**
Test-II (Aq. extract -400mg/kg)	102.83±6.93**	154.66±5.06**	143.16±4.36**	123.66±5.73**
Standard (Glibenclamide) (5mg/kg)	104.17±4.57**	143.16±7.09**	135.33±5.92**	131±3.48**

Values expressed as mean±S.D, n=6 One-way ANOVA followed by Dunnett's test.

Data represents mean ± S.D (n=5); *p<0.05 less significant as compared to normal control ;p<0.01significant as compared to Alloxan control ; ***p<0.001 more significant as compared to Alloxan control ;ns is no significant as compared to normal control.**

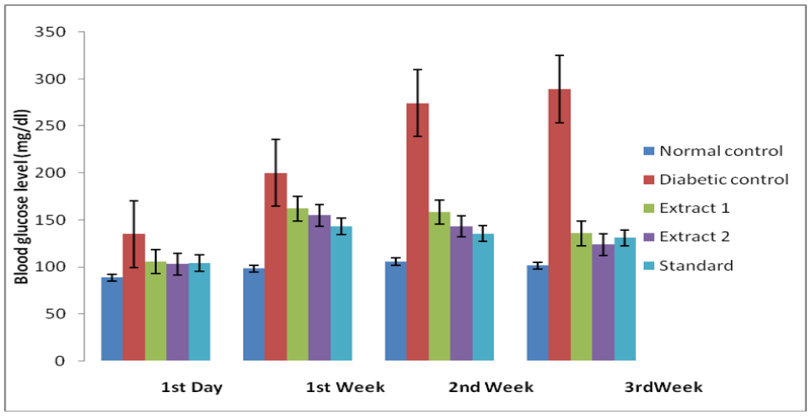


Figure 1: Effect of aqueous leaf extract of *Cansjera rheedii* on fasting blood glucose level in Alloxan induced diabetic rats

NC- Normal control; DC-Diabetic Control ;Ext1-Extract 1 [Test-I (Aq. extract -200mg/kg)];Ext2-Extract 2 [Test-II (Aq. extract - 400mg/kg)

Table 6: Effect of aqueous extract of leaves of *Cansjera rheedii* on cholesterol

GROUPS	CHOLESTEROL (mg/dl)
Normal control (0.5% CMC)	104±1.50
Diabetic control (150mg/kg ALLOXAN)	130.33±1.50
Test-I(Aq. extract -200mg/kg)	120±1.19**
Test-II (Aq. extract - 400mg/kg)	113.66±2.01**
Standard drug Glibenclamide) (5mg/kg)	101.55±1.03**

Values expressed as mean±S.D, n=6 One-way ANOVA followed by Dunnett's test. **p<0.01significant as compared to Alloxan control.

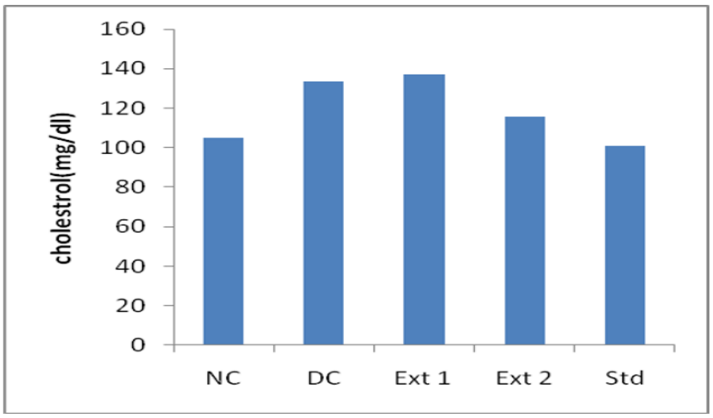


Figure 2: Effect of aqueous extract of leaves of *Cansjera rheedii* on cholesterol.

NC- Normal control ; DC-Diabetic Control ; Ext1-Extract 1 [Test-I (Aq. extract -200mg/kg)] ; Ext2-Extract 2 [Test-II (Aq. extract - 400mg/kg)

Table 7: Effect of Aqueous extract of leaves of *Cansjera rheedii* on Triglycerides

Groups	Triglyceride (mg/dl)
Normal control (0.5% CMC)	74.15±1.14
Diabetic control (150mg/kg ALLOXAN)	103.3±1.35
Test-I(Aq. extract -200mg/kg)	95.11±1.02**
Test-II (Aq. extract- 400mg/kg)	80.5±0.86**
Standard drug Glibenclamide) (5mg/kg)	80.16±1.09**

Values expressed as mean±S.D, n=6 One-way ANOVA followed by Dunnett's test.p<0.01significant as compared to Alloxan control.**

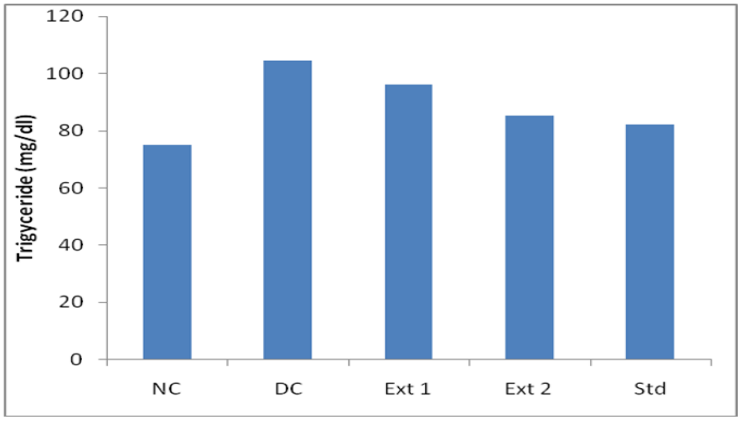


Figure 3: Effect of Aqueous extract of leaves of *Cansjera rheedii* on Triglyceride

Table 8: Effect of Aqueous extract of leaves of Cansjera rheedii on total proteins

Groups	Total protein (g/dl)
Normal control (0.5% CMC)	7.3±0.12
Diabetic control (150mg/kg ALLOXAN)	3.13±0.45
Test-I(Aq. extract -200mg/kg)	4.13±020*
Test-II(Aq. extract - 400mg/kg)	5.10±0.21*
Standard drug Glibenclamide) (5mg/kg)	4.63±0.30**

Values expressed as mean±S.D, n=6 One-way ANOVA followed by Dunnett's test ; *p<0.05 significant as compared to normal control. ; **p<0.01significant as compared to Alloxan control.

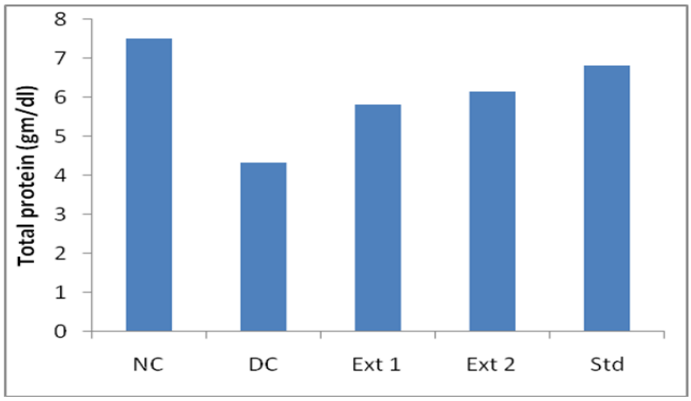


Figure 4: Effect of Aqueous extract of leaves of *Cansjera rheedii* on total proteins.

Table 9: Effect of Aqueous extract of leaves of *Cansjera rheedii* on SALPSALP-Serum Alkaline Phosphatase**

Groups	SALP (IU/L)
Normal control (0.5% CMC)	264.25±2.19
Diabetic control (150mg/kg ALLOXAN)	401.16±0.78
Test-I(Aq. extract -200mg/kg)	306.33±0.28**
Test-II(Aq. extract - 400mg/kg)	259±1.56**
Standard drug Glibenclamide) (5mg/kg)	308.07±1.12**

Values expressed as mean±S.D,n=6 One-way ANOVA followed by Dunnett's test.p<0.01significant as compared to Alloxan control.**

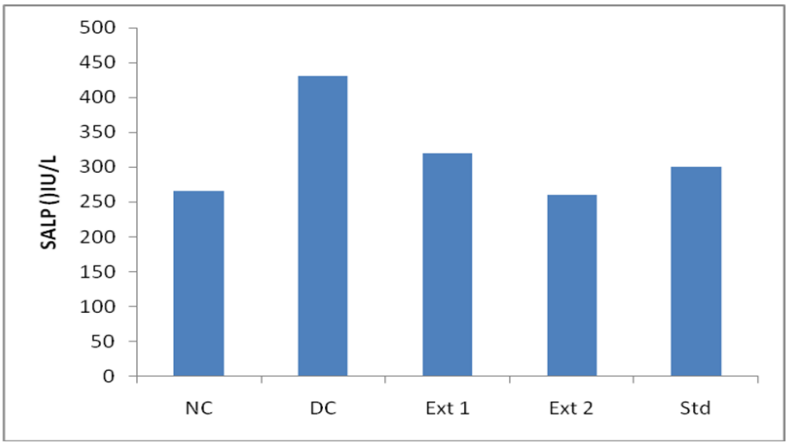


Figure 5: Effect of Aqueous extract of leaves of *Cansjera rheedii* on SALP

Table 10: Effect of aqueous extract of leaves of *Cansjera rheedii* on SGOTSGOT- Serum glutamic oxaloacetic transaminase**

Groups	SGOT(IU/L)
Normal control (0.5% CMC)	33.07±1.97
Diabetic control (150mg/kg ALLOXAN)	85.43±1.65
Test-I(Aq. extract -200mg/kg)	54±1.14**
Test-II(Aq. extract - 400mg/kg)	47.53±1.15**
Standard drug Glibenclamide) (5mg/kg)	35±1.67**

Values expressed as mean±S.D, n=6 One-way ANOVA followed by Dunnett's test.p<0.01significant as compared to Alloxan control.**

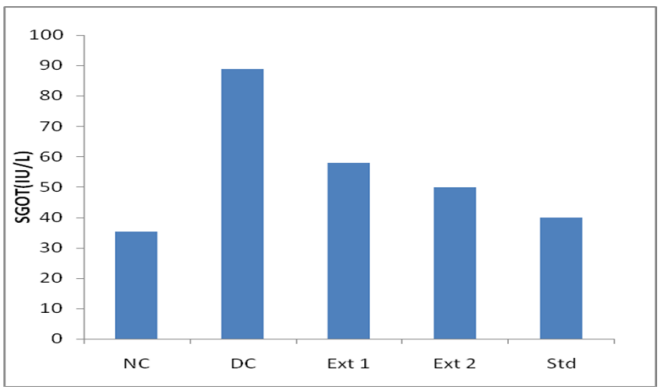


Figure 6: Effect of aqueous extract of leaves of *Cansjera rheedii* on SGOT

Table 11: Effect of aqueous extract of leaves of *Cansjera rheedii* on SGPTSGPT- Serum glutamic pyruvic transaminase**

Groups	SGPT(IU/L)
Normal control (0.5% CMC)	64.16±1.24
Diabetic control (150mg/kg ALLOXAN)	109.17±1.57
Test-I(Aq. extract -200mg/kg)	84.10±1.06**
Test-II(Aq. extract - 400mg/kg)	63.23±1.27**
Standard drug Glibenclamide) (5mg/kg)	60±1.03**

Values expressed as mean±S.D, n=6 One-way ANOVA followed by Dunnett's test. **p<0.01significant as compared to Alloxan control.

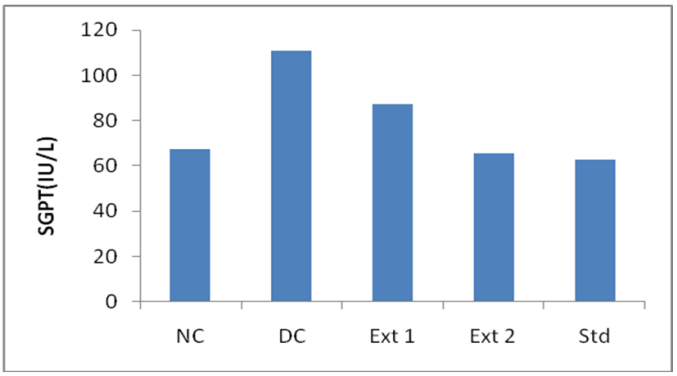


Figure 7: Effect of aqueous extract of leaves of Cansjera rheedii on SGPT

The aqueous extracts of *Cansjera rheedii* did not have any toxic effects up to1000mg/kg dose. The animals were alive healthy and active during the observation period.

The results demonstrated in (Table 5) showed that the lower dose of aqueous leaf extract 200mg/kg produced a statistically significant reduction in fasting blood glucose level on the 28th day, when compared with diabetic controlled animal whereas the higher dose of leaf extract, 400mg/kg produced a statistically significant reduction ($p<0.05$) from the 14th day and a significant reduction ($p<0.01$) from 21st days treatment the leaf extract was found to be more potent at the higher dose, 400mg/kg and it brought down the elevated blood glucose level in alloxan induced diabetic rats nearer to the normal range when compared to Standard Glibenclamide (5mg/kg)(Table 5,Fig 1).

The lower dose (200mg/kg) and higher dose(400mg/kg) of leaf extract brought down the cholesterol,SALP,SGOT,SGPT and Total protein levels($p<0.01$) in alloxan induced diabetic rats nearer to normal range when compared to Standard Glibenclamide (5mg/kg). The lower dose (200mg/kg) showed more potent reduction in cholesterol,SALP,SGOT,SGPT and Total protein levels when compared to higher dose (400mg/kg)(Tables 6 to 11) (Fig 2 to 7).

Antioxidant activity

The *in-vitro* antioxidant activity of ethanolic and aqueous extracts of leaves of *Cansjera rheedii* was evaluated and the results obtained are as follows,

DPPH scavenging activity [12]

Different concentrations ethanolic and aqueous extracts (25, 50, 75, 100,250 µg/mL) of test and standard (ascorbic acid) compounds were prepared in methanol solution and added (3.0 mL) to the DPPH solution (1.0 mL, 0.1 mM) and allowed to stand for 30 minutes in dark. The free radical scavenging activity was determined by measuring the decrease in absorption at 517 nm in a UV-visible spectrophotometer. The actual decrease in absorption was measured against that of the control (0.2 mM, DPPH solution).

Both ethanolic and aqueous extracts of *Cansjera rheedii* showed DPPH scavenging activity at 25µg/ml and it increased with increase in concentration. The percentage DPPH scavenging activity of ethanolic and aqueous extracts from 25 to 250µg/ml was 19.28±2.18 to 72.78±1.003 and 19.46±0.507 to 62.90±2.85 respectively. At 250µg/ml ethanolic extract of *Cansjera rheedii* produced DPPH scavenging activity comparable ($p<0.01$) to that of ascorbic acid. [Table No.12, Figure No. 8]

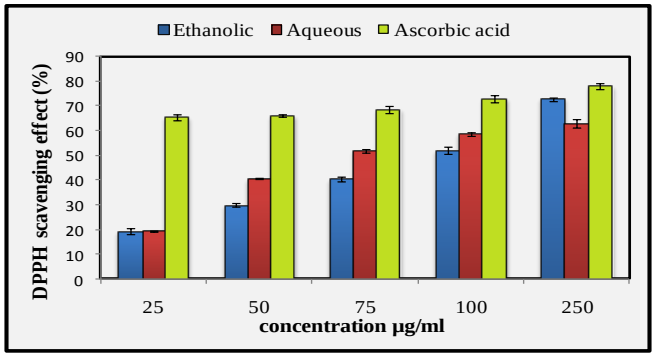


Figure 8: Values are percentage DPPH radical scavenging activity, mean ± SEM, *Cansjera rheedii* ethanolic and aqueous extracts, and ascorbic acid.

Table 12: Percentage DPPH radical scavenging activity of ethanolic and aqueous extracts of *Cansjera rheedii* .

Test compound	Concentrations (µg/ml)				
DPPH	**25**	**50**	**75**	**100**	**250**
E1	19.28±2.18	29.93±1.15	40.51±1.93	52.21±2.66	72.78±1.00^{*a**c}
E2	19.46±0.50	40.66±0.63^{**b}	52.08±1.28^{**b}	58.78±1.21^{**b}	62.90±2.85^{**a}
AA	65.43±1.86	66.11±0.62	68.60±2.58	72.90±2.45	77.97±2.08

Values are percentage DPPH radical scavenging activity, mean ± SD, E1 and E2– *Cansjera rheedii* ethanolic and aqueous extracts respectively, AA- Ascorbic acid *-p<0.05, **-p<0.01, 'a' indicates comparison of both the extracts with std-AA, 'b' indicates comparison of aqueous with ethanolic extract, 'c' indicates comparison of ethanolic with aqueous extracts.

CONCLUSION

The present study brings about a conclusion that aqueous extract of *Cansjera rheedii* showed potent hypoglycemic activity .The antioxidant activity of ethanolic extract of *Cansjera rheedii* was found to have better (p<0.01) DPPH scavenging activity when compared to aqueous extracts of *Cansjera rheedii* at 250µg/ml.

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No conflict of interest declared.

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