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Antioxidant and Antidiabetic Activity of *Decalepis Hamiltonii* Leaves Extract

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ABSTRACT

In most nations, diabetes mellitus is a serious and rising health issue. The goal of the study was to compare the antidiabetic effects of leaves extracts of Decalepis hamiltonii in normal and diabetic rats induced with alloxan. Using 120 mg/kg of alloxan monohydrate, diabetes was induced. In order to considerably lower blood glucose levels in both normal and alloxan-induced diabetic rats, D. hamiltonii methanolic extract was given at doses of 200 mg and 400 mg, along with glibenclamide at a dose of 7 mg/kg bwt. Additionally, the injection of the extract dramatically reduced the levels of AST, ALT, triglycerides, and total blood cholesterol while simultaneously raising the amount of liver glycogen. In order to perform the oral glucose tolerance test (OGTT), separate groups were given doses of D. hamiltonii methanolic extract (200 mg, 400 mg), and glibenclamide (7.5 mg), which effectively reduced blood sugar levels following an oral glucose load at all time points. These findings indicate that diabetic rats' blood glucose levels were successfully lowered by the methanolic extract of D. hamiltonii leaves. The antioxidant potential of the leaves was measured using various in vitro assays. The methanolic extracts showed high antioxidant activity measured as scavenging of DPPH, superoxide and hydroxyl radicals. The antioxidant activity did not correlate with the phenolic content of the extracts. These results demonstrate the antioxidant potency of the leaves extracts which could be the basis for its alleged health promoting potential of D. hamiltonii. The leaves of D. hamiltonii could serve as a new source of natural antioxidants or nutraceuticals with potential applications to reducing the level of oxidative stress and related health benefits.

Key words: Decalepis hamiltonii, Antidiabetic, Antioxidant, Alloxan, AST, OGTT

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic condition characterized by impaired insulin production, either hereditary or acquired, and by diminished organ response to produce insulin. Diabetes mellitus is a disease that is initially identified by a loss of glucose homeostasis brought on by errors in insulin production. These errors also result in insulin action that impairs the metabolism of both glucose and other fuels that provide energy, such as lipids and proteins. End-stage renal disease, cardiomyopathy and heart attacks, strokes, retinal degeneration resulting in blindness, and non-traumatic amputations are all significantly increased by 2 DM. The primary risk factor for cardiovascular and cerebrovascular illnesses is dyslipidemia, which is relatively frequent in diabetes people. ²⁻⁴

A number of methods are currently available to reduce hyperglycemia, including insulin therapy, which increases glucose utilization while decreasing glucose production, but has a number of side effects, including insulin resistance, anorexia nervosa, brain atrophy, and fatty liver, after long-term use; treatment with sulfonylurea, which encourages pancreatic islet cells to secrete insulin; metformin, which lowers hepatic glucose production; and α -glucos.⁵ Finding new classes of molecules is crucial to finding solutions to these issues because all of current treatments, unfortunately, have low effectiveness and a variety of negative effects. Despite the availability of well-known anti-diabetic medications on the market, therapies made from medicinal plants are successfully utilized to treat this condition.⁶

During the early stages of research on experimental diabetes, alloxan was one of the most frequently utilized chemical diabetogens. It is a 2,4,5,6-tetra-oxo-hexa hydro pyrimidine analogue of the cyclic urea molecule. Alloxan causes diabetes in mice and hinders the pancreatic islet of Langerhans cells' ability to secrete insulin in response to glucose. According to reports, alloxan accumulates quickly and preferentially in cells as opposed to non-cells. Numerous investigations suggest that alloxan has an impact on the membrane potential and ion channels in cells, either directly or indirectly.⁷

An antioxidant's primary property is its capacity to snare free radicals. The atomic and molecular forms of oxygen, also known as reactive oxygen species, are the most harmful free radicals. (ROS). These free radicals can cause degenerative disorders by oxidizing lipids, proteins, and nucleic acids. Inhibiting oxidative pathways, antioxidants scavenge free radicals such peroxide, hydroperoxide, and lipid peroxyl. The study of plant-based antioxidants generated from fruits, nuts, oils, and vegetables is now attracting a lot of interest from academics.⁸⁻⁹

The objective of the current study is to evaluate the antioxidant activity of *D. hamiltonii* leaves that were cultivated and collected during a two-year period. In addition to determining antioxidant activity, antioxidant molecules such phenols, flavonoids, minerals, like selenium, zinc etc. were also analyzed. Rats with normal and alloxan-induced diabetes were given methanolic extracts of the leaves of *D. hamiltonii* to test for antidiabetic efficacy.

2. MATERIALS AND METHODS

2.1 Chemicals

Alloxan monohydrate was purchased from Oxford Lab Fine Chem LLP, Mumbai. Glibenclamide was purchased from Johnlee Pharmaceuticals Pvt. Ltd. Mumbai. Ascorbic acid; aluminum chloride, 2, 2' - azino-bis-(3- ethylbenzothiazoline-6sulphonic acid) (ABTS); ferric chloride (FeCl₃); Folin-Ciocalteu; bovine serum albumin (BSA); potassium persulphate; 2,2'diphenyl-1-picrylhydrazyl (DPPH); nitro blue tetrazolium (NBT); phenazine methosulphate (PMS); reduced glutathione (GSH); 1,2dithio-bis nitro benzoic acid (DTNB); sulphosalicylic acid; thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Oxford Lab Fine Chem LLP, Mumbai. Sulphuric acid; 2-deoxyribose; riboflavin; sodium carbonate (Na₂CO₃); sodium hydroxide (NaOH); sodium nitrite (NaNO2); disodium hydrogen phosphate (Na₂HPO₄) and hydrogen peroxide (H₂O₂) were obtained from Sigma Aldrich Chemicals Pvt Ltd, Delhi. Potassium ferricyanide [K₃Fe (CN)₆]; triflouroacetic acid; sodium dihydrogen phosphate (NaH₂PO₄) and all solvents n-hexane (99.8%); chloroform (99.8%); ethyl acetate (99.8%) and *n*-butanol (99.8%) used were of analytical grade and distilled deionized water (dd. H₂O) was prepared by Ultrapure TM water purification system Sigma Aldrich Chemicals Pvt Ltd, Delhi.

2.2 Plant Material

Leaves of plant *D. hamiltonii* was purchased from India Mart. The leaves were washed and excess of water was drained off and dried on filter paper. Shade dried plant material was crushed in electrical mix grinder to a fine powder, and it was further used for the studies.

2.3 Phytochemical Screening

The methanolic extracts of leaves of *D. hamiltonii* were subjected to preliminary phytochemical tests to detect the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins, terpenoids, coumarins, carbohydrates and flavonoids using standard techniques.

2.4 Animals

The study employed adult Wistar rats (180-250 g) of both sexes. The animals were kept in cages and housed under the same 12:12 h light cycle, with free access to water. The Animal Ethics Committee of the College granted permission and approval for the animal research.

2.5 Determination of Antioxidant Activity

The evaluation of the antioxidant activity was carried out by free radical scavenging method (DPPH and ABTS) and ferric reducing antioxidant power (FRAP).

2.5.1 DPPH Scavenging Activity (DPPH)

The antiradical power of substances was measured by the decrease of absorption of DPPH (1,1-Diphenyl-2- picrylhydrazyl). To 950 μl of a methanol solution of DPPH (0.1 mM) were added to 50 μl of the plant leaves extract. After 30 min, the absorbance of the mixture was measured at 517 nm. The ability to scavenge DPPH radical was calculated using the following formula.

% Inhibition of DPPH =
$$\frac{A_{c-}A_{s}}{A_{c}}X$$
 100

 A_c = Absorbance of control A_s = Absorbance of sample

2.5.2 ABTS Scavenging Activity (ABTS)

The technique is based on the scavenging of ABTS*+ [(3-ethyl benzothiazoline 6-sulfonic acid) diammonium salt] radical cation which was generated by mixing solutions of ABTS (7 mmol/L) and potassium persulfate (2.45 mmol/L). The mixture was then incubated in the dark at room temperature for 16h. The product was diluted for optimal absorbance of 0.7 at 734 nm. The decolorization of the ABTS*+ solution by 100 µg/mL of the test sample or reference compound (Trolox) was monitored by a decrease in absorption at 734 nm during 30 min. The antioxidant activity expressed in µM trolox equivalent antioxidant capacity (TEAC)/ mg dry weight (DW).

2.5.3 Ferric Reducing Antioxidant Power (FRAP)

The method is based on reduction of ferric tripyridyl triazine (Fe³ – TPTZ) to ferrous complex tripyridyl triazine (Fe² – TPTZ) by an antioxidant in acidic pH. The ferrous Fe (II) complex -TPTZ develops a blue colour with maximal absorbance at 593 nm. The methodology of Benzie and Strain20 was used. FRAP mixture consists of 10 parts of acetate buffer solution (300 mM) at pH 3.6, 1 volume of 10 mmol/l 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l HCl and 1 volumes of a solution of FeCl₃.6H₂O (20 mM). To 2 ml of the FRAP mixture were added 10 μ l of the plant extract. After incubation of 15 min at room temperature, the absorbance was measured at 593 nm. The calibration range was prepared with Trolox. Results are expressed as μ mol Trolox equivalent antioxidant capacities (TEAC)/mg DW [172].

2.6 Antidiabetic Activity

2.6.1 Preparation of Extract

The leaves of *D. hamiltonii* was dried in the shade and ground to a coarse powder. The powder was defatted in petroleum ether (60-80 °C) before being extracted with 90% methanol in a soxhlet extractor. The solvent was evaporated and dried in vacuum under reduced pressure, and the filtrate obtained was used for further research.

2.6.2 Animals

The current study used healthy albino wistar rats weighing 150-200 g. They were housed in polypropylene cages under temperature control (25±2 °C) with 12-hour light-dark cycles. Before the study, all of the animals were acclimatized for 7 days. They were fed a standard pellet diet and water. All studies were approved by the Animal Ethical Committee.

2.6.3 Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test was performed on normal rats that had been fasted overnight (18 hours). Four groups of six rats each were formed from the rats. Group 1 served as the normal control and received 0.3% Carboxy methyl cellulose orally. Glibenclamide was administered orally to Group 2 at a dose of 7 mg/kg bwt. Groups 3 and 4 were given orally 200 mg and 400 mg/kg of *D. hamiltonii methanolic* leaves extract dissolved in 0.3% Carboxy methyl cellulose, respectively. After 30 minutes of treatment, all groups were given 2 g/kg of glucose orally. Blood samples were taken just before glucose administration, as well as 30, 60, 120, and 150 minutes later. A commercial kit was used to measure blood glucose levels.

2.6.4 Hypoglycemic activity in normal rats

Fasted overnight, healthy wistar albino rats weighing 150-200 g were divided into four groups of six rats each.

- A normal control received 0.3% Carboxy methyl cellulose orally in Group 1.
- Glibenclamide (7 mg/kg bwt) was administered orally to normal rats in Group 2.
- Normal rats were given an orally methanolic extract of D. hamiltonii leaves (200 mg/kg bwt) dissolved in 0.3% Carboxy methyl cellulose in Group 3.
- Normal rats were given an orally methanolic extract of D. hamiltonii (400 mg/kg bwt) dissolved in 0.3% Carboxy methyl cellulose in Group 4.
- Blood samples were collected before and after treatment at 1, 2, and 4 hours, and the glucose level was determined using a commercial kit.

2.6.5 Induction of Diabetes

In overnight fasted rats, 150 mg/kg of alloxan monohydrate dissolved in normal saline was administered intraperitoneally to induce diabetes. ¹⁰ The animals were fed standard pellets and water ad libitum after 1 hour. Blood glucose levels were measured after 72 hours, and rats with blood glucose levels greater than 180 mg/dl were chosen for the study.

2.6.6 Hypoglycemic Activity in Diabetic Rats

After fasting overnight, healthy wistar albino rats weighing 150-200 g were divided into five groups of six rats each.

- Group 1: A normal control was given 0.3% Carboxy methyl cellulose orally.
- Group 2: Diabetic rats were given Alloxan monohydrate (150 mg/kg bwt) orally.
- Group 3: Diabetic rats were given the reference drug Glibenclamide (7 mg/kg bwt) orally.
- Diabetic rats were given an orally administered methanolic extract of *D. hamiltonii* (200 mg/kg bwt) dissolved in 0.3% Carboxy methyl cellulose.
- Diabetic rats were given an orally administered methanolic extract of *D. hamiltonii* (400 mg/kg bwt) dissolved in 0.3% Carboxy methyl cellulose.
- Blood samples were taken before, one, two, and four days after treatment, and the glucose level was determined using a commercial kit.

2.6.7 Biochemical Analysis

After fasting overnight, the rats were sacrificed by cervical decapitation, and blood samples were collected to clot and serum separated by centrifugation at 2500 rpm for 10 minutes. The following parameters were measured: serum glucose, total cholesterol, triglycerides, aspartate transaminase (AST), and alanine transaminase (ALT). The Oxidase method was used to calculate serum glucose. The serum AST and ALT activities were determined using the Reitman and Frankel method. Total cholesterol and triglycerides were calculated using the appropriate method. The liver was dissected and washed in normal saline before being stored at 80 °C for glycogen content analysis using Anthrone reagent.

2.6.8 Statistical Analysis

The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by the student's test. The values are mean \pm SD for six rats in each group. Statistical significance was determined at p <0.05.

3. RESULT AND DISCUSSION

3.1 Phytochemical Test

Phytochemical analysis of *D. hamiltonii* aqueous leaf extract indicated the presence of alkaloids, tannins, flavonoids, sterols, terpenoids, and saponins. All of these types of chemicals have been shown to have significant biological activity.

Table 1: Preliminary Phytochemical Screening of D. hamiltonii

-	GL 1	E.4	I	D 4	CI I C
S.	Chemical	Etha	Aqueous	Pet.	Chlorofo
No	Constituents	nolic	riqueous	Ether	rm
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Steroids	+	+	+	+
5	Flavonoids	+	+	+	+
6	Saponins	+	+	+	+
7	Fixed oils and fats	-	-	-	-
8	Tannins	+	+	+	+
9	Proteins and amino acids	-	-	+	-
10	Terpenoids	-	-	-	-

Abbreviations: (+) is positive; (-) is Negative

3.2 Acute Toxicity Test

There was no mortality in mice after taking the aqueous extract orally, even at dosages as high as $5\,000\,\text{mg/kg}$, indicating that the oral LD50 was more than $5\,000\,\text{mg/kg}$.

Table 2: LD50 and ED50 of D. hamiltonii

Plant Name	LD 50	ED 50
D. hamiltonii	5000 mg/kg	200mg/kg

3.3 Determination of Antioxidant Activity

Table 3 shows the antioxidant activity evaluated by three techniques. The IC50 of the DPPH radical scavenging was 126.6 \pm 1.07 µg/ml respectively for the plants leaves. For the ABTS test, leaves exhibited values of 1432.06 \pm 20.41 µM TEAC/mg DW respectively. Whether for the DPPH test or the ABTS test, differences between the male and female trees were not significant. With respect to FRAP, the MEOAs of leaves recorded significantly the values of 62.50 \pm 4.52 µM TEAC/mg DW. The literature reports that the antioxidant activity of plant extracts depends on

both the nature of the test and the phenolic compounds. For example, quercetin showed very low antioxidant activity in the FRAP test but very high activity in the DPPH and ABTS assays. Indeed, polyphenols are compounds which have more than one hydroxyl group attached to one or more benzene rings. They are usually encountered as esters or glycosides rather than as free compounds. Then, the antioxidant activity of the polyphenols depends on the arrangement and the number of hydroxyl groups in the phenolic rings and their connections with the saccharides. Polyphenols could act as reducing initiators, chelating agents or by the prevention of oxidative reactions caused by active singlet oxygen. For our study, the antioxidant activity could be explained by the presence of cardenolide, triterpenoids, pregnanes, carbohydrate, phenolic acids, flavonoids, and amino acids. The previous chemical studies deduced that D. hamiltonii is a very rich plant with different compounds, including flavonoids triterpenes, pregnanes, cardenolides, cardiac glycosides, and carbohydrate. Finally, due to the presence of a single hydroxyl in the B ring. These data make it possible to conclude that the antioxidant activity recorded in our work results from the synergy of the various phenolic compounds, even with other non-phenolic antioxidants.

Table 3: Antioxidant activity of *D. hamiltoni* leaves extract assessed by DPPH, ABTS and FRAP methods

Activity	DPPH (%)	IC ₅₀ (μg/ml)	ABTS (μM TEAC/ mg DW)	FRAP (µM TEAC/mg DW)
D. hamiltoni	81.53 ± 0.62	126.6 ± 1.07	1432.06 ± 20.41	62.50 ± 4.52

Values are expressed in mean \pm SEM. Means in each column followed by different letters are significantly different (P<0.05).

3.4 Antidiabetic Activity

Serum glucose, total cholesterol, triglycerides, aspartate transaminase, and alanine transaminase levels were significantly higher in the diabetic control rats compared to the non-diabetic control group.

3.4.1 Effects of MEDH in Oral Glucose Tolerance in Normal Rats

Table 4 shows the blood glucose levels after oral administration of glucose (2 g/kg) of the control, Glibenclamide (7 mg/kg), and methanolic extract of D. hamiltonii (200 mg and 400 mg/kg) at various time points (0, 30, 60, 120, and 150 minutes).

All groups experienced a peak increase in blood glucose at 30 minutes. When compared to the control group, the Glibenclamide and 400 mg MEDH treated group had lower blood glucose levels at 150 minutes.

3.4.2 Effect of MEDH on Blood Glucose Level in Normal Fasted Rats

Table 5 shows the blood glucose levels in euglycemic rats after 0, 1, 2, and 4 hours of administration. Glibenclamide (7 mg/kg) and methanolic extract of D. hamiltonii (200 mg and 400 mg/kg) administration to euglycemic rats was not significant at 1 h, but was significant at 4 h (p < 0.05) compared to control.

3.4.3 Effect of MEDH on Blood Glucose Level in Alloxin Induced Diabetic Rats

Table 6 shows the blood glucose levels in normal and diabetic rats after 0, 1, 2, and 4 hours of administration. When compared to normal control rats, diabetic rats had significantly higher blood glucose levels. Glibenclamide (7 mg/kg) and methanolic extract of D. hamiltonii (200 mg and 400 mg/kg) administration reduced blood glucose in diabetic rats compared to control rats. The fourth day of Glibenclamide and 400 mg of MEDH treatment resulted in a significant hypoglycemic effect in the diabetic group.

3.4.4 Effect of MEDH on Serum AST and ALT and Liver Glycogen in Alloxan Induced Diabetic Rats

The levels of serum AST and ALT, as well as liver glycogen, were shown in Table 7 for normal and experimental rats. When diabetic control rats were compared to non-diabetic control rats, there was a significant increase in serum AST and ALT and a decrease in liver glycogen content. Glibenclamide (7 mg/kg) and methanolic extract of D. hamiltonii (200 mg and 400 mg/kg) administration significantly reduced AST and ALT levels and increased glycogen content in diabetic rats compared to control rats.

3.4.5 Effect of MEDH on Serum Cholesterol and Triglycerides Alloxan Induced Diabetic Rats

When diabetic rats were compared to control rats, there was a significant increase in cholesterol and triglycerides. Glibenclamide (7 mg/kg) and methanolic extract of D. hamiltonii (200 mg and 400 mg/kg) restored cholesterol and triglyceride levels to near normal in rats.

Table 4: Effect of MEDH in oral glucose tolerance in normal fasted rats

		Blood Glucose level mg/dl (Mean±SD)					
S. No.	Group	Fasting	Post treatment				
			30 min	60 min	90min	120min	
1	Control (0.3%CMC)	73.48 ± 0.62	75.56 ± 0.82	73.28 ± 1.05	71.55 ± 1.01^{a}	72.52 ± 0.58^{a}	
2	Glibenclamide (7mg/kg) + glucose	77.26 ± 1.12	194.93 ± 1.19	142.51 ± 0.58	98.47 ± 1.23 ^b	76.7 ± 0.71^{a}	
3	MEDH (200 mg/kg) + glucose	78.13 ± 0.78	198.67 ± 1.11	149.72 ± 1.24	103.87 ± 1.93^{b}	92.71 ± 1.04 ^b	
4	MEDH (400 mg/kg) + glucose	71.70 ± 0.89	192.12 ± 0.89	153.87 ± 1.09	92.11 ± 1.45 ^b	77.21 ± 0.72^{a}	

Values are expressed as mean \pm SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at $P \le 0.05$.

Table 5: Effect of MEDH on blood glucose level in normal rats

		Blood Glucose level mg/dl (Mean±SD)			
S. No.	Group	Fasting	Time (h) after treatment		
		Fasting	1	2	4
1	Control (0.3%CMC)	71.02 ± 0.48	69.15 ± 0.60	67.86 ± 0.58	70.46 ± 0.85^{a}
2	Glibenclamide (7mg/kg)	75.23 ± 0.67	65.23 ± 0.70	57.35 ± 0.60	53.55 ± 0.84^{b}
3	MEDH (200 mg/kg)	69.16 ± 1.14	66.24 ± 0.52	61.22 ± 0.47	$57.23 \pm 0.82^{\circ}$
4	MEDH (400 mg/kg)	74.13 ± 1.17	64.12 ± 0.50	56.63 ± 0.57	51.15 ± 0.95^{b}

Values are expressed as mean \pm SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at $P \le 0.05$.

Table 6: Effect of MEDH in blood glucose levels in alloxan induced diabetic rats

		Blood Glucose level mg/dl (Mean±SD)				
S. No.	Group	Fasting	Time (h) after treatment			
		T usting	1	2	4	
1	Control (0.3%CMC)	71.38 ± 1.08	71.34 ± 2.42	71.04 ± 1.17	70.76 ± 1.63^{a}	
2	Diabetic control (Alloxan)	202.18 ± 1.53	205.12 ± 1.64	208.32 ± 1.16	206.9 ± 1.36^{b}	
3	Diabetic + Glibenclamide (7mg/kg)	200.12 ± 0.87	187.46 ± 0.79	172.05 ± 3.24	160.25 ± 2.77°	
4	Diabetic + MEDH (200 mg/kg)	201.7 ± 1.09	188.28 ± 1.21	175.44 ± 2.23	$157.63 \pm 1.86^{\circ}$	
5	Diabetic + MEDH (400mg/kg)	202.13 ± 0.88	185.15 ± 1.77	155.83 ± 1.73	150.81 ± 1.38^{d}	

Values are expressed as mean \pm SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at $P \le 0.05$.

Table 7: Effect of MEDH on serum AST and ALT and liver glycogen in alloxan induced diabetic rats

S. No.	Group	AST (IU/L)	ALT (IU/L)	Glycogen (g/100g)
1	Control (0.3%CMC)	46.04 ± 2.3^{a}	53.55 ± 3.29^a	$3.6\pm0.15^{\rm a}$
2	Diabetic control (Alloxan)	189.71 ± 5.47^{b}	184.17 ± 2.44^{b}	0.73 ± 0.032^{b}
3	Diabetic + Glibenclamide (7mg/kg)	$78.07 \pm 2.56^{\circ}$	$86.9 \pm 2.87^{\circ}$	$3.08 \pm 0.13^{\circ}$
4	Diabetic + MEDH (200 mg/kg)	118.76 ± 2.85^{d}	121.5 ± 2.86^{d}	1.9 ± 0.11^{d}
5	Diabetic + MEDH (400mg/kg)	80.8 ± 2.64^{c}	$91.06 \pm 1.90^{\circ}$	$2.5 \pm 0.21^{\circ}$

Values are expressed as mean \pm SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at $P \le 0.05$.

Table 8: Effect of MEDH on serum cholesterol and triglycerides in alloxan induced diabetic rats

S. No.	Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)
1	Control (0.3%CMC)	125.15 ± 6.11 ^a	147.82 ± 3.56^{a}
2	Diabetic control (Alloxan)	259.81 ± 6.75^{b}	233.6 ± 5.56^{b}
3	Diabetic + Glibenclamide (7mg/kg)	130.13 ± 5.01^{a}	162.4 ± 4.51°
4	Diabetic + MEDH (200mg/kg)	$159.73 \pm 7.65^{\circ}$	$183.5 \pm 6.17^{\rm d}$
5	Diabetic + MEDH (400mg/kg)	142.05 ± 4.45^{d}	165.4 ± 5.23°

Values are expressed as mean \pm SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at $P \le 0.05$.

4. CONCLUSION

On the basis of above results it is concluded that selected medicinal plant extracts, fractions and most of the sub-fractions showed moderate to high antioxidant activities while the pure isolated compounds from these fractions were found to be very weak antioxidants, except 5-O-caffeoyl quinic acid which was isolated from the active fraction of D. hamiltoni leaves and exhibited the potent antioxidant activity towards DPPH free radical. The antioxidant capacity of these plants revealed due to the emergence of bioactive composition, which are promising source of natural antioxidants and can be exploited for multiple industrial and domestic applications. Our study identified presence of cardenolide, triterpenoids, pregnanes, carbohydrate, phenolic acids, flavonoids, and amino acids. The previous chemical studies deduced that D. hamiltoni is a very rich plant with different compounds, including flavonoids triterpenes, cardenolides, cardiac glycosides, and carbohydrate. The same was true for the antioxidant activity except for the FRAP test which showed a greater activity in the flowers. In conclusion, methanolic extract of D. hamiltonii leaves contains antihyperglycemic agents capable of lowering blood glucose levels and having a hypolipidemic effect.

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