Research Article

Antioxidant and Antidiabetic Activities of Ethanolic Extract of *Hibiscus sabdariffa* calyx and *Stevia rebaudiana* Leaf

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ABSTRACT

The prevalence of diabetes mellitus has increased tremendously in developing countries of tropical regions. The need for an effective and safe alternative is of importance as oral hypoglycaemic drugs have reported to cause side effects on prolonged use. In the present study two tropical plants, *Hibiscus sabdariffa* calyx and *Stevia rebaudiana* leaves were assessed for their *in vitro* antioxidant and antidiabetic activities. Total phenol and flavonoids of ethanolic extracts were quantified. Bioactive compounds were screened by Gas Chromatography Mass Spectrometry (GC-MS) analysis. Antioxidant activity was assessed by 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and superoxide radical scavenging assays. Antidiabetic ability was evaluated by inhibition of enzymes involved in carbohydrate metabolism. Glucose uptake by 3T3-L1 cells was also assessed. Ethanolic extract of Stevia leaf (EtSL) had a higher percentage of phenol and flavonoids. The GC-MS analysis showed the presence of phytochemicals like Phenol-2,4-bis (1,1-dimethylethyl) in Roselle calyx and 6-methoxyflavone in Stevia leaves. The antioxidant assessment showed that EtSL significantly inhibited superoxide radical (IC_{50}=45.81±1.21 µg/mL). The antidiabetic assessment revealed that Ethanolic extract of Roselle calyx (EtRC) showed strong inhibition of alpha amylase (IC_{50}=27.95±0.32 µg/mL) and alpha glucosidase (IC_{50}=6.21±0.18 µg/mL). Glucose uptake in 3T3-L1 cells demonstrated that both plants had significant insulin sensitizing ability. It was also revealed that the phenol and flavonoid content strongly correlated with IC_{50} values of antioxidant and antidiabetic assays. Results of this study show that both plants are a potential source of bioactive phytochemicals and can be used as a plant-based antioxidant and antidiabetic agent.

Key words: Tropical plants, *Hibiscus sabdariffa, Stevia rebaudiana*, Antioxidant activity, Antidiabetic activity.

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INTRODUCTION

Diabetes mellitus is one of the most prevalent metabolic disorder. Impaired macronutrient metabolism in diabetes leads to oxidative stress by increasing the free radical production. Oxidative stress increases the severity of complications and incidence of death by diabetes.[1] In spite of great development in research prevalence of diabetes has increased worldwide, especially in tropical developing countries. Synthetic oral hypoglycaemic drugs along with insulin are used in the management of diabetes. However, prolonged use of these drugs have caused side effects, therefore there is a need for an effective and safe alternative form of treatment.[2] Secondary metabolites like phenols and flavonoids present in medicinal plants have the ability to act as antioxidants and can be used in prevention and management of diseases like diabetes.[3]

Plants grown in tropical zone contribute to majority of medicinal plants as they are a rich source of bioactive compounds when compared to plants grown in temperate zone.[4] One such plant which has been of

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relevance is Roselle *(Hibiscus sabdariffa)*. Roselle is an annual herbaceous plant belonging to the family of Malvaceae is cultivated both in tropical and subtropical zones of Asia, Central America and Africa. Presence of various phytochemicals in the calyces of Roselle attribute to its medicinal value. Major phytochemicals in red calyces are anthocyanins, apart from this organic acids, amino acids, minerals and phenolic compounds are also present which have the ability to modulate, antioxidant mechanisms and inhibit enzymes of lipid and carbohydrate metabolism involved in diabetes and obesity.[5]

Another tropical plant of great medicinal value is *Stevia rebaudiana*. Stevia is a perennial shrub native to South America, belongs to the Asteraceae family. Stevia leaves are used as non-caloric sweeteners as it contains diterpene glycosides, which are about 300 times sweeter than saccharose. Apart from being used as a natural sweetener, leaves also contains phenols and flavonoids which has the ability to act as plant based antidiabetic alternative.[6,7]

Therefore, in the present study antioxidant and antidiabetic potential of two tropical plants Roselle (calyx) and Stevia (leaves) was assessed. Total phenolic and flavonoid content along with GC-MS analysis was also done to investigate the presence of various chemical constituents. Correlation between phenolic compounds and antioxidant and antidiabetic activity of the plant extracts was also studied.

MATERIALS AND METHODS

Plant collection and crude extract preparation

Roselle calyces and stevia leaves were procured from farms in Karnataka and Madhya Pradesh, India. Then, calyces and leaves were cleaned, shade dried and powdered using an electric blender. Maceration method was used for the extraction. About 500 g of plant powder was weighed and soaked in 250 mL of 95% ethanol and was left to macerate for 72 hr. Then the supernatant was filtered through Whatman's filter No.41. and condensed in a hot plate at 40°C. Extracts were then stored in a freezer at -20°C until further analysis.

Determination of total phenol and flavonoid content

Total phenolic content was determined by Folin-Ciocalteu method with slight modifications.[8] In alkaline medium phenols present in plant extracts, reacts with phosphomolybidic acid in Folin- Ciocalteau reagent to form molybdenum blue complex.[9] Change in absorbance was measured at 765 nm using a UV-vis spectrophotometer. Standard curve was calibrated using different concentrations of gallic acid. The mean of three readings was used and the total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100 g of extract.

Total flavonoid content of the extract was determined by the aluminium chloride ($AlCl_3$) colorimetric method with modifications. The keto or hydroxyl group of flavonoids reacts with $AlCl_3$ to form acid stable complexes.[10] Standard curve was calibrated using different concentrations of quercetin. The mean of three readings was used and the total flavonoid content was expressed as mg of quercetin equivalents (QE)/100 g of extract.

GC-MS analysis

Ethanolic extract of Roselle calyx (EtRC) and Stevia leaves (EtSL) were analysed using a Perkin Elmer GC–MS (Model Perkin Elmer Clarus 500). Compounds were detected using an electron ionization system with ionization energy 70 eV. Helium was used a gas carrier with flow rate of 1 ml/min. The diluted extract (1/100, v/v) was injected into the injector with split mode (ratio- 1:120).[11] National Institute Standard and Technique (NIST) library was used to analyse the mass spectrum GC-MS.

Assessment of antioxidant activity by DPPH and Superoxide anion radical scavenging assay

DPPH radical scavenging assay was performed according to Baba and Malik were antioxidants present in the extracts reduces the purple chromatogram of DPPH to yellow hydrazine[12] and decrease in absorbance was measured using a UV- vis spectrophotometer at 570 nm. Superoxide anion radical scavenging assay was determined according to Gulcin *et al.*[13] Non-enzymatic ethylenediaminetetraacetic acid -nicotinamide adenine dinucleotide (EDTA/ NADH) system produces superoxide radical which reduces nitro blue tetrazolium (NBT) to a purple formazan.

The percentage of inhibition of free radical was calculated using the following formula:

Inhibitory activity (%) = [(OD control – OD sample) / OD control] × 100%

Assessment of antidiabetic activity by inhibition of alpha amylase, alpha glucosidase and dipeptidyl peptidase IV (DPP-IV) enzymes

Inhibition of alpha amylase activity was assessed by method of Ou *et al.*[14] with modifications. The α-glucosidase inhibitory activity was assessed according to the method of Roy and Pushpa[15] by estimating the release of yellow coloured 4- nitrophenol from

p-nitrophenyl α-D glucopyranoside. DPP-IV inhibitory activity was determined by method of Konrad *et al.*[16] Enzyme inhibition is observed by decreased formation of pNA yellow from Gly-Pro p-nitroanilide. Acarbose was used as the standard for alpha amylase and glucosidase inhibition assay and vildagliptin was used as the standard for DPP-IV inhibition assay. Inhibitory activity was calculated using the formula:

Inhibitory activity (%) = [(OD control – OD sample) / OD control] × 100%

Assessment of antidiabetic activity by *in vitro glucose uptake*

Cell culture

3T3-L1 mouse pre-adipocytes were procured from ATTC, USA. Cells were cultured at 37°C in Dulbecco's modified eagle medium (DMEM) containing 4.5 g/L D-glucose with 10% heat-inactivated foetal bovine serum (FBS), 1% penicillin and 1% hepes buffer at a humidified atmosphere containing 5% CO_2. Pre-adipocytes were differentiated into adipocytes by the method given by Moon *et al.*[17]

Glucose uptake by 3T3-L1 adipocytes

Insulin-sensitizing effect of the extracts was assessed by glucose uptake assay. Glucose uptake into 3T3-L1 adipocytes was carried according to the method of Park *et al.*[18] Differentiated cells were treated with various concentrations of EtRC and EtSL (6.2–500 μg/mL) and 500 μg/mL of Metformin (positive control) for 48 hr. The decrease in fluoresce of 2-NBDG after uptake into cells was measured at an excitation wavelength of 485 nm and emission wavelength of 535 nm using a fluorescent micro-plate reader.

Statistical analysis

All the tests were performed in triplicates and the results were expressed as mean ± SD. One-way analysis of variance (ANOVA) was performed and the means were separated using Tukey's pair wise analysis and Pearson's correlation coefficient was also calculated. The p-values at $P<0.05$ were considered as statistically significant. All statistical analysis was performed using the statistical tool Graph pad Prism 5.

RESULTS

Total phenol and flavonoid content

The results of phytochemical estimation revealed that the extracts had good levels of phenols and flavonoids (Figure 1). The total phenol (514.22±1.6 mg GAE/g) and flavonoid (160.76±1.09 mg QE/g) content of Stevia leaf extract was significantly higher than Roselle calyx extract.

GC-MS Analysis

The bioactive principles with their molecular formulae, retention time (R_t), molecular mass and peak area by GC-MS analysis is represented in Table 1 and Table 2. A total of 9 and 11 compounds were identified in EtRC and EtSL respectively. Compounds like Phenol-2,4-bis (1,1-dimethylethyl) in Roselle calyx and Morin, 6-methoxyflavone in Stevia leaves were identified and these compounds have known to have therapeutic potential.

Assessment of antioxidant activity by DPPH and Superoxide anion radical scavenging assay

Table 3 shows the antioxidant activity of extracts. Both EtRC and EtSL were able to significantly scavenge and reduce free radicals with the increase the concentration of extracts. Among both plants, EtSL had significantly higher inhibition of free radicals at the maximum concentration of 120 μg/mL and had a significantly low IC_{50} value compared to EtRC (Table 4).

Assessment of antioxidant activity by inhibition of alpha amylase, alpha glucosidase and DPP-IV enzymes

Inhibitory activity of ethanolic extracts and standard drugs against enzymes involved in carbohydrate metabolism is represented in Table 5. EtRC and EtSL showed good inhibition of alpha amylase with a IC_{50} of 27.95±0.32 μg/mL and 28.12±0.04 μg/mL respectively. Against alpha glucosidase, EtRC and Acarbose demonstrated similar IC_{50} values of 6.21±0.18 μg/mL and 6.94±1.17 μg/mL. Whereas, IC_{50} value of EtSL (29.38±0.68 μg/mL) was a significantly low compared

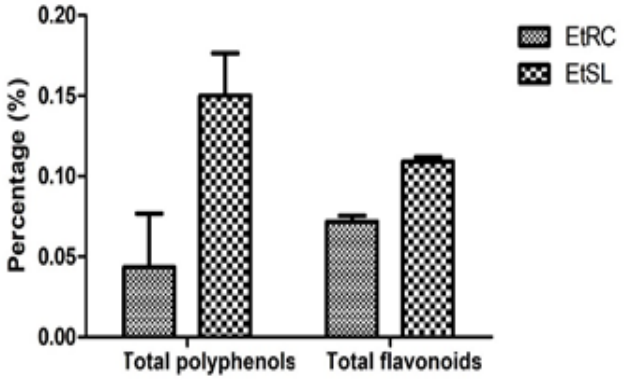

Figure 1: Total phenol and flavonoid content of EtRC and EtSL.
Mean ±Standard deviation of three independent estimations; EtRC – Ethanolic extract of Roselle calyx; EtSL - Ethanolic extract of Stevia leaves

to standard drug Vildagliptin against DPP-IV enzyme (Table 4).

Glucose uptake by 3T3-L1 adipocytes

The results of glucose uptake activity were compared with standard drugs like insulin and metformin (insulin-sensitizer). Figure 2. Shows the glucose uptake potential of the extracts. At a concentration of 500 µg/mL, the uptake of glucose by Roselle calyx and Stevia leaves extract treated groups showed significant an increase of 48.81± 3.45% and 43.0± 2.24%, respectively, compared to the control group (12.44±1.24%) (p<0.05). Whereas, insulin (100 nM) and metformin (500 µg/mL) enhanced the glucose uptake by 64.36±2.89 % and 63.32 ± 1.11% respectively.

Correlation between phytochemical content and antioxidant and antidiabetic activity

Phenol content of EtRC had a strong negative correlation with the IC_{50} values of superoxide radical scavenging assay (R=-0.7669). Whereas, flavonoids had a negative correlation with the IC_{50} values of DPPH and Superoxide assays (R=-0.96137 and R=-0.22883) indicating that the flavonoids present in Roselle

Table 1: Bioactive compounds identified in ethanolic extract of Roselle calyx.

S.NO	R_T (Min)	Compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1	11.72	Cyclohexanone, 2-(2-butynyl)-	$C_{10}H_{14}O$	150.22	6.62
2	16.95	2-Benzyl-2H-tetrazol-5-amine 5-amino-2-benzyltetrazole	$C_8H_9N_5$	175.19	24.86
3	18.68	Phenol-2,4-bis (1,1-dimethylethyl)	$C_{15}H_{24}O$	220.35	12.35
4	19.73	9,10-Anthracenedione-1,4-diamino-2,3 dihydro	$C_{14}H_{12}N_2O_2$	240.26	5.20
5	20.58	9 Oximino-2,7-diethoxyfluorene	$C_{17}H_{17}NO_3$	283.32	10.36
6	21.92	6-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	21.94
7	22.7	9,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	16.25
8	24.1	Elaidic acid, isopropyl ester	$C_{21}H_{40}O_2$	324.5	0.81
9	26.92	3,4-Dihydroxy-1,6-bis(4-methoxyphenyl) hexa-2,4-diene-1,6-dione	$C_{20}H_{18}O_6$	354.4	1.21

Table 2: Bioactive compounds identified in ethanolic extract of Stevia leaves.

S.NO	RT (Min)	Compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1	14.88	Cyclohexanol,1methyl-4-(1-methlethylidene)-	$C_{10}H_{18}O$	154.25	5.14
2	15.6	Benzenamine,2-methoxy-4-nitro-	$C_7H_8N_2O_3$	168.15	3.27
3	16.43	2,4,6(3H)-Pteridinetrione,1,4a,5,8a tetrahydro-7-methyl	$C_7H_6N_4O_3$	194.15	2.24
4	16.88	5,10-Undecadienoic acid, 2-methylene-, methyl ester	$C_{13}H_{20}O_2$	208.3	26.54
5	17.08	Danthron	$C_{14}H_8O_4$	240.21	9.34
6	17.72	Z-8-Hexadecene	$C_{16}H_{32}$	224.42	6.54
7	18.82	6-methoxyflavone	$C_{16}H_{12}O_3$	252.26	26.91
8	19.05	1,3,3-Trimethyl-1-(2'-hydroxyphenyl) indan-6-ol)	$C_{18}H_{20}O_2$	268.3	3.17
9	20.4	Methanone(2,4-dihydroxy-6-methylphenyl) (2-hydroxy-4-methoxy-6-methylphenyl)-	$C_{14}H_{12}O_4$	288.29	19.62
10	21.43	Morin	$C_{15}H_{10}O_7$	302.23	3.73
11	25.3	Calusterone	$C_{21}H_{32}O_2$	316.5	1.86

calyx, contributed to the antioxidant activity more when compared to phenols. However, in EtSL both phenols and flavonoids did not show correlation with the scavenging activity, indicating that, the presence of other phytochemicals attributes to its antioxidant activity. Phenol content also showed strong negative correlation with alpha amylase (R=-0.85646) and DDP-IV (R=-0.83577) inhibition activities of EtRC. Whereas total flavonoid content showed negative correlation with alpha glucosidase inhibition activity (R=-0.80718). In EtSL, phenols showed strong negative correlation with alpha amylase activity (R=-0.99925) and flavonoid showed a weak negative correlation (R=-0.26616). This shows that increase in phytochemical content contributes to lower IC_{50} value of Roselle calyx and Stevia leaves.

DISCUSSION

Presence of phytochemicals in adequate levels in diet can contribute to various health benefits. Major phytochemicals, phenols and flavonoids have antioxidant, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties with the ability to regulate several cellular enzymes. This is suggested to help in the prevention and management of diabetes, obesity, inflammation, cardiovascular and neurological disorders.[19,20] The results of present study revealed that both plants had good levels of these phytochemical with phenol and flavonoid content of Stevia leaves being significantly higher compared to Roselle calyx. This result was in

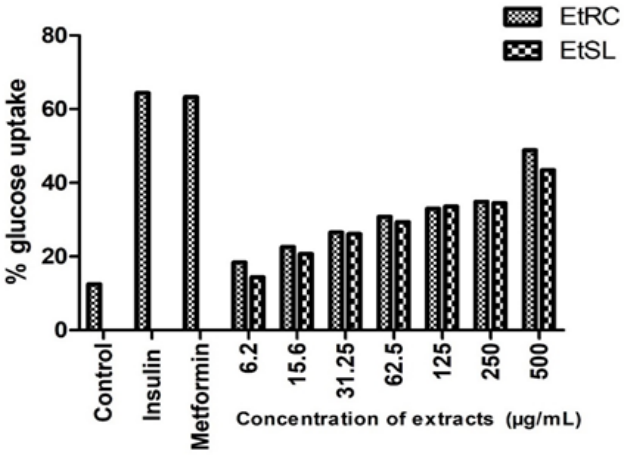

Figure 2: Uptake of Glucose in 3T3 Cells by EtRC and EtSL. Mean ±Standard deviation of three independent estimations; EtRC – Ethanolic extract of Roselle calyx; EtSL Ethanolic extract of Stevia leaves

Table 3: Antioxidant activities of different extracts of Roselle calyx and Stevia Leaves.

Concentration (µg/mL)	DPPH (% inhibition)		SRSA (% inhibition)	
	EtRC	EtSL	EtRC	EtSL
20	19.88±0.99^{a}	25.06±0.62^{a}	41.85±0.95^{a,*}	33.48±0.69^{a}
40	43.82±0.90^{b}	31.1±1.58^{b}	50.07±1.60^{b}	43.67±1.14^{b}
60	48.28±0.493^{c}	44.39±0.62^{c}	50.52±2.15^{b}	56.76±1.84^{c}
80	50.43±0.76^{c,d}	74.06±0.77^{d,*}	52.66±1.15^{b,c}	74.27±0.95^{d}
100	53.29±0.83^{d,e}	79.49±0.85^{d,e}	57.68±1.39^{c,d}	79.29±1.14^{d,e}
120	65.43±0.65^{f}	82.26±0.72^{f,*}	79.12±1.49^{e,*}	83.25±1.14^{e,f}

Mean ±Standard deviation of three independent estimations; $^{a-f}$Values followed by the different superscripts are significantly different within the column; *Values are significantly different within the row, p<0.05; SRSA - Superoxide Radical Scavenging Assay; EtRC – Ethanolic extract of Roselle calyx; EtSL - Ethanolic extract of Stevia leaves.

Table 4: Antioxidant and Antidiabetic activity of Roselle calyx and Stevia Leaves expressed as IC_{50} values (µg/mL).

Plant Extract/ Standard	DPPH inhibition	SRSA inhibition	Alpha amylase inhibition	Alpha glucosidase inhibition	DPP-IV inhibition
EtRC	62.13±0.63	59.44±2.49	27.95±0.32	6.21±0.18	220.22±0.47
EtSL	67.59±0.95	45.81±1.21*	28.12±0.04	33.70±1.0*	29.38±0.68*
Acarbose	-	-	3.86±0.05*	6.94±1.17	-
Vildagliptin	-	-	-	-	126.27±1.94

Mean ±Standard deviation of three independent estimations; *Values are significantly different within the row, p<0.05 EtRC – Ethanolic extract of Roselle calyx; DPP-IV- Dipeptidyl peptidase IV; EtSL - Ethanolic extract of Stevia leaves

Table 5: Antidiabetic activities of different extracts of Roselle calyx and Stevia Leaves.

Concentration (µg/mL)	Alpha amylase (% inhibition)			Alpha glucosidase (% inhibition)			DPP-IV (% inhibition)		
	Acarbose	EtRC	EtSL	Acarbose	EtRC	EtSL	Vildagliptin	EtRC	EtSL
6.2	80.15±1.17^{a,*}	12.24±0.49^{a,}	16.15±1.91^{a}	45.41±0.50^{a}	49.85±0.61^{a,*}	21.62±0.53^{a}	4.41±0.11^{a}	3.78±0.58^{a}	41.32±3.70^{a,*}
15.6	82.06±0.95^{a,b,*}	30.48±0.38^{b}	37.88±0.90^{b}	67.24±0.54^{b,*}	53.39±0.56^{b}	37.16±0.09^{b}	13.24±0.56^{b}	3.96±0.72^{a}	40.86±1.72^{a,*}
31.25	83.52±2.11^{b,*}	55.88±0.66^{c}	55.55±0.09^{c}	87.62±0.59^{c,*}	58.38±0.72^{c}	46.37±1.41^{c}	25.39±0.32^{c}	4.90±0.44^{a,b}	53.19±1.27^{b,*}
62.5	86.64±2.56^{b,c,*}	61.00±0.63^{d}	60.24±1.60^{d}	90.49±0.31^{c,d,*}	62.92±0.92^{d}	74.02±1.30^{d}	36.69±1.25^{d}	16.95±0.68^{c}	56.13±0.70^{b,c,*}
125	90.25±0.14^{d,*}	71.12±0.45^{e}	71.12±0.61^{e}	92.02±0.06^{d,e,*}	66.27±0.49^{e}	77.53±1.04^{d,e}	49.51±0.76^{e}	24.93±0.68^{d}	62.92±0.45^{a,*}
250	91.36±0.47^{d,e,*}	68.42±0.52^{f}	74.79±0.95^{e,f}	93.43±0.31^{e,*}	76.71±0.35^{f}	81.09±1.68^{e,f}	65.76±1.01^{f}	56.76±0.12^{e,*}	64.67±1.39^{d,e}
500	94.83±3.98^{f}	73.70±0.10^{g}	81.45±1.12^{g,*}	95.16±0.18^{e,f,*}	78.96±0.22^{f,g}	91.90±0.51^{g}	72.65±0.75^{g}	59.24±1.11^{e,f}	77.56±0.67^{f}

Mean ±Standard deviation of three independent estimations; $^{a-g}$Values followed by the different superscripts are significantly different within the column; *Values are significantly different within the row, $p<0.05$; EtRC – Ethanolic extract of Roselle calyx; EtSL Ethanolic extract of Stevia leaves

agreement with the findings of Periche *et al.*[21] where total phenol and flavonoid content in the infusion of Stevia leaves was reported to be 90 mg GAE/g and 56 mg catechin equivalents. Whereas, in Roselle calyx flavonoid content (44.76±0.72 mg QE/g) was higher than the total phenolic content (19.92±0.100 mg GAE/g). Similar findings were reported by Okereke *et al.*[22] where flavonoids and phenol content were 20.08% and 1.10%. The bioactive compounds identified by GC-MS in EtRC were Phenol-2,4-bis (1,1-dimethylethyl) (12.35%) and 9 Oximino-2,7-diethoxyfluorene (10.36%). Previous studies have reported that Phenol-2,4-bis (1,1-dimethylethyl) has antioxidant and antidiabetic activity. [23,24] Studies also show that 9 Oximino-2,7-diethoxyfluorene is an antidiabetic and antimicrobial agent[25] and 9,11-Octadecadienoic acid, methyl ester has been used in the management of sickle cell anemia.[26]

In EtSL, bioactive compounds identified were 6-methoxyflavone (6.54%), Cyclohexanol, 1 methyl-4-(1-methlethylidene)- (5.14%) and Morin (3.73%). Previous studies have reported Cyclohexanol, 1 methyl-4-(1-methlethylidene)- to have antioxidant and anticancer activity.[27,28] 6-methoxyflavone and Morin are important flavonoid have been reported to have various biological activities like anticancer and anti-inflammatory ability.[29,30]

Medicinal plants contain natural antioxidant compounds which have the ability to prevent oxidative stress related diseases like diabetes by delaying the oxidation of macromolecules like carbohydrates and lipids. [31] Among both extracts, EtSL significantly had higher inhibition of DPPH radical compared to EtRC at the concentration of 120 µg/mL. Whereas, EtSL (45.81±1.21 µg/mL) was able to inhibit super oxide radical with a significantly lower IC_{50} value when compared to EtRC (59.44±2.49 µg/mL). Overall, both plant extracts exhibited good reducing and free radical scavenging activity. Antioxidant activity of the extracts can be attributed to the phytochemicals present in them. Similar findings were reported on the antioxidant activity of Roselle calyx and Stevia leaves.[32-34]

Inhibition of enzymes involved in hydrolysis of carbohydrate can reduce post prandial glucose levels and can be used as a strategy to manage type 2 diabetes mellitus.[35] Therefore, ability of Roselle calyx and Stevia leaves to inhibit alpha-amylase and alpha- glucosidase enzymes were assessed. Both the extracts showed inhibition of alpha amylase in a dose dependent manner. Highest percent (81.45± 1.12) of inhibition of alpha amylase was seen at a concentration of 500 µg/mL of EtSL compared to EtRC (73.30± 0.10%). The result revealed that as the concentration of test extracts increased, it exhibited increasing alpha Glucosidase inhibitory activity. Both extracts showed good inhibitory effects on both carbohydrate digesting enzymes. In addition, IC_{50} values of EtRC was also comparable to standard drug Acarbose. Therefore, it can be inferred that Roselle calyx can be used in the management of postprandial hyperglycaemia.

Incretins, Glucagon-like-Peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP) play an important role in glucose homeostasis by increasing the secretion of insulin. DPP-IV is an intestinal enzyme, which degrades GLP-1 and GIP and inactivates them to decrease insulin secretion thereby contributes to hyperglycaemia.[36] The findings of this study revealed excellent inhibitory effect of EtRC and EtSL extracts on DPP-IV enzyme, and its potential use as an effective alternative for management of type 2 diabetes. Both extracts exhibited good inhibition of DPP-IV enzyme with maximum inhibition of 59.21±1.11% (EtRC) and

77.56±0.67% (EtSL) at 500 µg/mL. Stevia leaves was able to inhibit DPP-IV more significantly than Roselle calyx ($P<0.05$).

Phenols and flavonoids regulate glucose metabolism through various mechanisms by acting as inhibitors, insulin sensitizers, insulin mimetics and insulin secretagogues.[37] The results of present study shows that both the extracts exert insulin sensitizing effect in a dose-dependent manner. Amongst both the extracts, EtRC showed significantly higher uptake of glucose into the cells when compared to EtSL. In a study by Prata *el al*.,[38] glycosides from Stevia leaves also exhibited glucose uptake potential by acting as insulin mimetic in rat cardiac fibroblasts.

CONCLUSION

Growing prevalence of diabetes in tropical countries was impetus to the conduction of the present study. Two plants (Roselle calyx and Stevia leaves) grown in the tropical region were assessed for their phytochemical constituents, antioxidant and antidiabetic activity. Stevia leaves had high levels of phenol and flavonoid compared to Roselle calyx. The GC-MS analysis of the plant extracts showed the presence bioactive compounds, which have the ability to act as natural antioxidant and antidiabetic agents. The results also revealed that Roselle calyx had better ability to inhibit carbohydrate digesting enzymes compared to Stevia leaves. Both plants demonstrated significant insulin sensitizing ability in 3T3-L1 cells. The results of this study strongly suggest that Stevia leaves and Roselle calyx have excellent antioxidant and antidiabetic properties and therefore can be used as a natural, plant based, safe and affordable alternative to synthetic drugs used in the management of diabetes mellitus.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

EtRC: Ethanolic extract of Roselle calyx; **EtSL:** Ethanolic extract of Stevia leaves; **GC-MS:** Gas chromatography-mass spectroscopy; **GAE:** gallic acid equivalents; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl.

SUMMARY

Ethanolic extract of both plants had good antioxidant and antidiabetic activity. Roselle calyx and Stevia leaves are therefore a potential source of phytochemicals, which can be used in the treatment of diabetes.

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