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## **Antihyperglycemic Activity of Leaf Essential Oil of *Citrus sinensis* (L.) Osbeck on Alloxan-Induced Diabetic Rats**

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### **Authors' contributions**

*This manuscript is a segment of masters research work of author OSO and was supervised by author NOM. Isolation of essential oil from Citrus sinensis leaf was carried out under the supervision of author LAU. The last author BPO assisted author OSO with the laboratory and statistical analysis and also with the drafting of this manuscript. All the authors have read and approved this manuscript.*

**Research Article**

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### **ABSTRACT**

**Aim:** The aim of this study was to investigate the antihyperglycemic activity of leaf essential oil of *Citrus sinensis* (Rutaceae) in alloxan –induced diabetic rats.

**Methodology:** Diabetes was induced in albino rats by intraperitoneal administration of single dose of alloxan monohydrate (150 mg/kg body weight). The leaf essential oil of *Citrus sinensis* at a dose of 110 mg/kg b.wt was administered every other day to the diabetic rats for a period of 15 days. The effects of leaf essential oil on blood glucose, hepatic glucose and glycogen were evaluated. 14.2 mg/kg body weight of metformin was used as a reference drug.

**Results:** Intraperitoneal administration of the oil to diabetic rats led to a significant reduction ( $P = .05$ ) of fasting blood glucose and hepatic glucose levels while hepatic glycogen significantly increased ( $P = .05$ ) when compared to diabetic control animals.

**Conclusion:** It is concluded that leaf essential oil of *Citrus sinensis* possesses significant antihyperglycemic effect on alloxan – induced diabetic rats at the dose tested.

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**Keywords:** *Citrus sinensis*; rutaceae; essential oil; antihyperglycemic; diabetic rats.

## 1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Diabetes results in abnormal levels of glucose in the blood stream. This can cause severe short-term and long term consequences ranging from brain damage to amputations and heart disease [1]. The incidence of diabetes has increased worldwide in recent years. The estimated number of people with diabetes was 30 million in 1985, 150 million in 2000 and then 246 million in 2007, according to the International Diabetes Federation. It expects this number to hit 380 million by 2025 [2]. Available treatments for diabetes mellitus are insulin, sulfonylureas, biguanides and glinides. Many of these treatment agents have a number of serious adverse effects such as hypoglycemia, drug-resistance, dropsy, and weight gain [3,4]. In view of this, the development of more effective and safer hypoglycemic agents derived from nature has become paramount.

Medicinal plants have gained importance for the treatment of Diabetes mellitus. Antihyperglycemic activity of most medicinal plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output, inhibit the intestinal absorption of glucose or facilitating metabolites in insulin dependent processes [5,6].

*Citrus sinensis* (Rutaceae) also known as Sweet orange is the most popular of the citrus fruits. It is widely cultivated in most regions of the world. The essential oils of *Citrus* are placed within the glands in the outer layer of the fruit skin. This oil is composed of many constituents, including monoterpenes, sesquiterpenes, alcohols, esters and aldehydes. Quantitative chemical analysis done on the peel essential oil of Venezuelan sweet orange (*Citrus sinensis* (L.) Osbeck) reveals the presence of 39 compounds with d-limonene as a major constituent (94.55%) [7]. Furthermore, another work on quantitative analysis of the volatile components of fruit peel of *Citrus sinensis* cv. *Valencia* that were isolated by Headspace solid phase microextraction (HS-SPME), single drop microextraction (SDME) and cold-press revealed that the oils were rich in monoterpenes: limonene (61.34, 68.27 and 90.5 %), as the major component, followed by myrcene (17.55, 12.35 and 2.50%); sabinene (6.50, 7.62 and 0.50%);  $\alpha$ -pinene (0.00, 6.65 and 1.40%) respectively [8]. As reported by [9], GC-MS analysis of leaf essential oil of *Citrus sinensis* var. *mosammi* revealed the oil to be a mixture of monoterpene (17.079%), sesquiterpenes (30.698%) and amides (2.339%). The sesquiterpenes constituted the major portion of essential oil.  $\beta$ -Phyllendrene (27.544%) was identified as a major sesquiterpene hydrocarbon followed by caryophyllene (3.154%) [9]. Among the monoterpenes, 3-carene was found as a major component (7.630%) followed by limonene (4.873%),  $\beta$ -Pinene (3.278%) and  $\alpha$ -Pinene (1.298%). Diethyltoluamide (2.339%) was also found in appreciable amount.

Leaf extracts of *C. sinensis* have been used in Nigerian local folk medicine to treat neurological disorders and to facilitate the digestion of food. It has also been used as an antidiabetic, antibacterial, antifungal, hypotensive, antioxidant, insect repellent, larvicidal, antiviral, uricosuric, anti-yeast, antihepatotoxic and antimutagenic agent [10-14]. In Odumara Obi-orodo, of Imo State, Nigeria, herbalists use concoction made from *Citrus sinensis* leaf, *Magnifera indica*, *Carica papaya*, *Psidium guajava* and other plants' leaf to treat malaria [15]. The oils are also generally in use in many foods, confectionary, drug, cosmetic and flavoring products [8]. There is paucity of scientific information on the glucose lowering

effect of the studied plant. The present study aims to investigate the antihyperglycemic activity of leaf essential oil of *Citrus sinensis* in alloxan-induced diabetic rats.

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

Alloxan monohydrate and dimethylsulfoxide (Sigma Chemical Company, St. Louis, Mo, USA), Accu-chek active glucometer and strips (Roche Diagnostic, Mannheim, Germany) and OHAUS analytical balance (Ohaus Corporation, NJ, USA), were used. Fresh leaves of *Citrus sinensis* were obtained from the Junior Staff quarters of the University of Ilorin, Nigeria where a voucher specimen of the plant was deposited. Identification of the leaf was carried out at the Plant Biology Department of the University of Ilorin. Albino rats (*Rattus norvegicus*) were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria.

### **2.2 Methods**

#### **2.2.1 Essential oil extraction**

Pulverished leaves of *Citrus sinensis* (800 g) were hydrodistilled for 3 hours in a Clevenger-type apparatus. Five (5) percent v/v of the resulting oil was prepared, using saline solution of dimethylsulphoxide (DMSO) [16].

#### **2.2.2 Gas chromatography/mass spectrometry (GC-MS) analyses**

A Hewlett-packard ITP5890A GC, interfaced with a VG analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were: ionization voltage 70eV, ion source 230°C. The GC was fitted with a 25 m × 0.25 mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15 µm. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the oils were computed in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectra with those of authentic samples and with data from literature.

#### **2.2.3 Experimental animals**

Forty (40) male albino rats of *norvegicus* strain (150–200 g) were housed in standard cages and allowed to acclimatize to animal house for 14 days. All rats were maintained under standard laboratory conditions (12-h light/dark cycle, 25±2°C). They were fed with standard rat chow and tap water *ad libitum*. Animals were then randomly selected into 4 groups (i.e. A, B, C and D) of 10 rats each representing (respectively) the Normal Control, Diabetic Control, Diabetic treated with 14.2 mg/kg b. wt. Metformin (reference drug) and Diabetic treated with 110 mg/kg b.wt. leaf essential oil of *Citrus sinensis*.

#### **2.2.4 Induction of experimental diabetes**

After fasting for 18 h, animals in the diabetic groups were subjected to a single intraperitoneal injection of freshly prepared 150 mg/kg body weight alloxan monohydrate freshly dissolved in sterile distilled water. 48 h after alloxan injection, fasting blood glucose (FBG) was determined using AccuChek active glucometer and compatible strips. Rats showing glucose concentration above 110 mg/dl were considered diabetic.

#### **2.2.5 Administration of oil**

All treatments were intraperitoneally (IP) administered to rats once daily as shown below:

- Group A (Normal control) received distilled water
- Group B (Diabetic control) received no treatment
- Group C (Diabetic + reference drug) was treated with 14.2 mg/kg body weight of metformin
- Group D (Diabetic + essential oil) was treated with 110 mg/kg body weight of leaf essential oil of *Citrus sinensis*

#### **2.2.6 Estimation of fasting blood glucose concentration**

Fasting blood glucose (FBG) concentration of all experimental groups were determined using a glucose oxidase-based commercial glucometer (AccuChek active, Roche Diagnostic) every other day of the experiment by withdrawing blood from the caudal vein of rat tail.

#### **2.2.7 Estimation of hepatic glucose and glycogen concentration**

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase [17]. The absorbance of the red-violet indicator from standard and samples were then read against reagent blank at 500 nm within 60 mins.

Glycogen measurement was based on the method described by [18]. Briefly, 100  $\mu$ l of diluted hepatic homogenate was hydrolyzed with 2M HCl and then heated for 2 hr at 95°C, followed by neutralization with 2M NaOH. The sample was analyzed and results expressed as glucose equivalents by determining glucosyl units using commercial kit for glucose medical diagnosis (RANDOX).

#### **2.2.8 Statistical analysis**

All data were expressed as the mean of five replicates  $\pm$  standard error of mean (S.E.M). Statistical evaluation of data was performed by SPSS version 16.0 using one way analysis of variance (ANOVA), followed by Duncan's multiple range test for multiple comparison. Values were considered statistically significant at  $P = .05$  (confidence level = 95%).

### 3 RESULTS AND DISCUSSION

#### 3.1 Results

##### 3.1.1 Chemical composition of leaf essential oil of *Citrus sinensis*

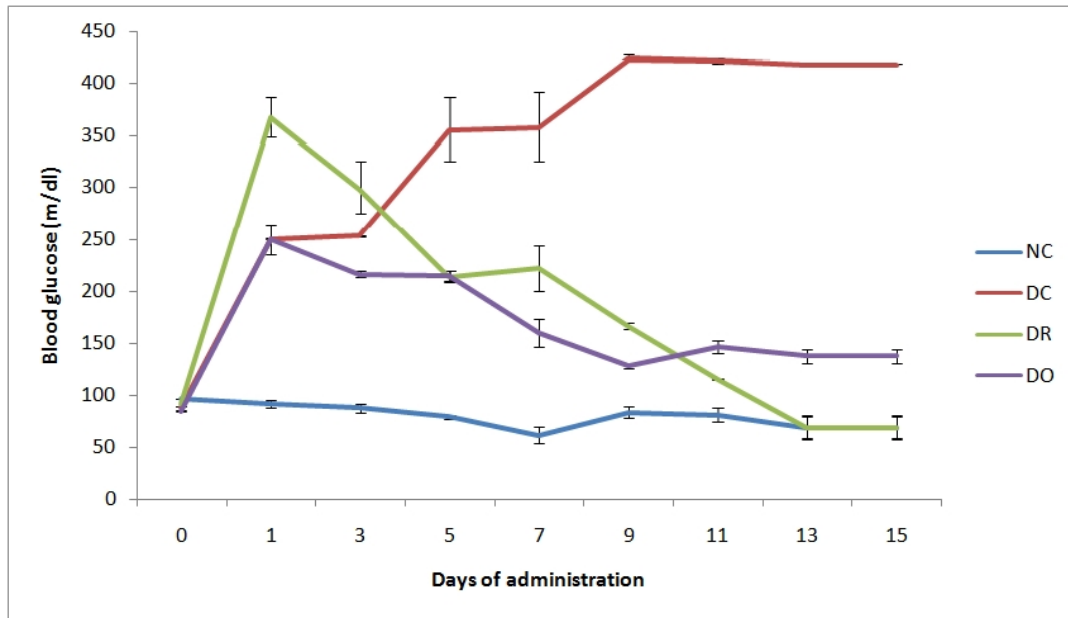
Table 1 shows the chemical composition of leaf essential oil of *Citrus sinensis*. The hydrodistillation of the leaves of studied plant generated oil (yield of 0.125%, w/v). Upon Gas Chromatography/Mass Spectrometry (GC/MS) analysis, the essential oil was found to contain 37 constituents. The major components of the oil were  $\beta$ -phylendrene, 3-Carene, D-Limonene and Caryophyllene.

**Table 1. Chemical composition of leaf essential oil of *Citrus sinensis***

s/n	Name of compound	Retention index	Area %
1	1R-alpha Pinene	937	1.07
2	Beta-Phellandrene	1053	22.85
3	Bicyclo(3..1.0)hexane,4-methylene-1-(methylethyl)	974	4.29
4	Beta-Pinene	990	1.39
5	Beta-mycrene	994	2.68
6	3-carene	1011	12.45
7	1,3-cyclohexadiene,1-methyl-4-(1-methylethyl)	1018	1.36
8	6-octenal,3,7-dimethyl	1159	1.28
9	3-cyclohexen-1-ol,4-methyl-1-(methylethyl)	1179	3.53
10	4-hexen-1-ol,5-methyl-2-(1-methylethyl)	1166	1.80
11	1,3,8-p-Menthatriene	1111	0.96
12	D-Limonene	1047	4.96
13	1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl)	1062	1.72
14	Cyclohexene,1-methyl-4-(1-methylethylidene)	1096	3.10
15	2,6-octadienal,3,7-dimethyl(E)	1320	2.46
16	Cyclohexane,1-ethenyl-1-methyl-2-,4-bis(1-methylethenyl)-, (1S-(1.alpha.,2.beta.))	1375	1.79
17	CaryoPhyllene	1467	2.15
18	2,6,9,11-Dodecatetraenal,2,6,10-trimethyl	1706	1.12
19	Pyridine,2-propyl	960	2.30
20	6-Octen-1-ol,3,7-dimethyl	1233	4.59
21	Benzene,1-methyl-4-(1-methylethyl)	1033	0.82
22	1,6-Octadiene-3-ol,3,7-dimethyl acetate.	1359	2.00
23	2,6-Octadien-1-ol,3,7-dimethyl(E)	1276	2.85
24	6-Octen-1-ol,3,7-dimethyl acetate	1354	0.89
25	8-Hydroxyquinoline	1359	0.89
26	Caryophyllene oxide	1606	2.22
27	alpha-phellandrene	1032	0.57
28	2,6-octadienal,3,7-dimethyl(Z)	1294	2.60
29	Apiol	1680	0.76
30	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methyl- (1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha)	1512	1.10
31	1H-Benzocycloheptene,2,4a,5,6,7,8-hexahydro-3,5,5,9- tetramethyl	1499	0.83
32	Santolina triene	908	0.53
33	Tricyclo(2.2.1.0(2,6))heptane,1,3,3-trimethyl	896	1.00
34	Benzene,1-methyl-2(1-methylethyl)	1011	1.63
35	Cyclohexene,3-methyl-6-(1-methylethylidene)	1086	0.57
36	Phenol,2-methyl-5-(1-methylethyl)	1298	0.54
37	2,6,9,11-Dodecatetraenal,2,6,10-trimethyl	1706	1.15
<b>Total</b>			<b>98.8</b>

### 3.1.2 Fasting blood glucose concentration

The effect of leaf essential oil of *Citrus sinensis* on fasting blood glucose concentration in albino rats is presented in Fig. 1. Fasting blood glucose levels of diabetic rats were significantly increased ( $P = .05$ ) when compared with those of the Normal control group. Administration of the oil and Metformin led to a significant decrease ( $P = .05$ ) in the fasting blood glucose level of treated animals when compared with the diabetic control group.

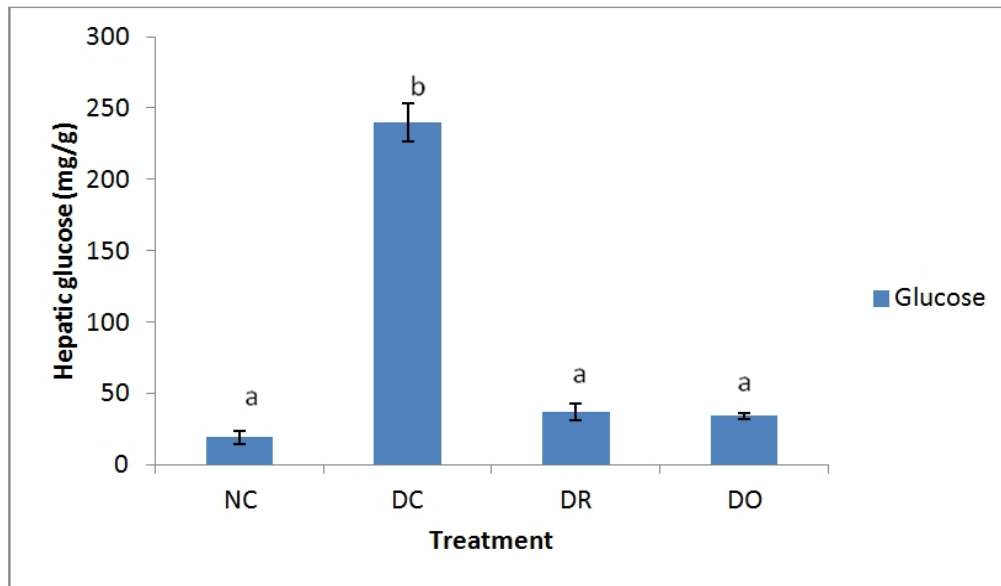


**Fig. 1. Antihyperglycemic effect of leaf essential oil of *C. sinensis* on fasting blood glucose level in alloxan-induced diabetic Rats**

Values are expressed as mean of five replicates  $\pm$  S.E.M, NC – Normal control; DC – Diabetic control; DR – Diabetic treated with reference drug; DO – Diabetic treated with leaf essential oil of *Citrus sinensis*, Day 0 values were the fasting blood glucose level before inducing diabetes in the experimental animals

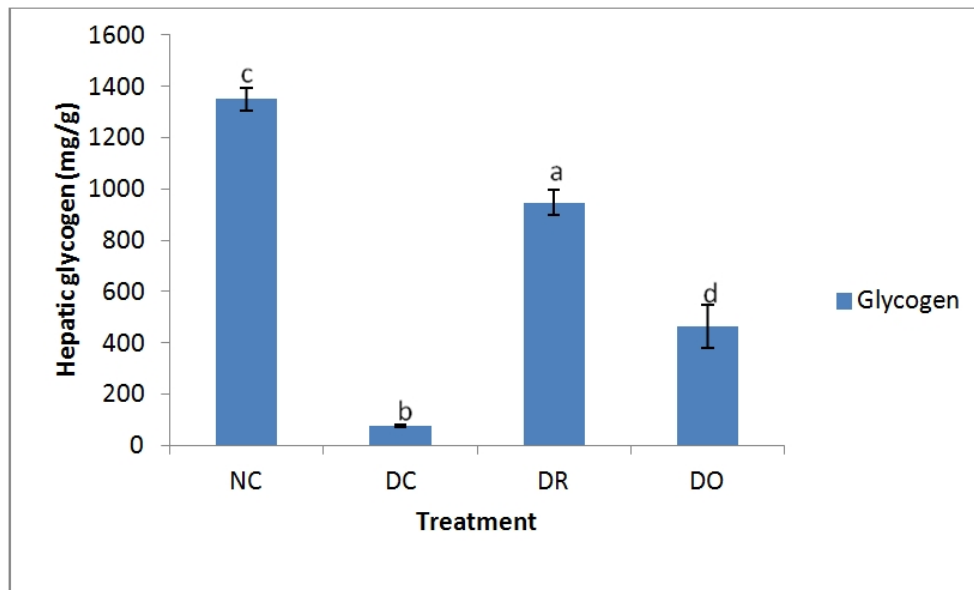
### 3.1.3 Hepatic glucose and glycogen

The diabetic control rats showed a significant increase ( $P = .05$ ) in hepatic glucose level when compared with the Normal control animals (Fig. 2). However, treatment with the essential oil and Metformin led to drastic significant decrease ( $P = .05$ ) in hepatic glucose levels of the experimental animals. There was no significant difference ( $P = .05$ ) in the hepatic glucose level of the animals treated with the essential oil and Metformin when compared to the normal control animals. The effect of the essential oil on hepatic glycogen of rats is presented in Fig. 3. The diabetic control animals showed a significantly reduced ( $P = .05$ ) hepatic glycogen concentration when compared to the Normal control group. Administration of essential oil and metformin significantly increased ( $P = .05$ ) the hepatic glycogen level of the treated experimental animals. However, neither metformin nor the essential oil was able to increase the hepatic glycogen level of the animals to normal levels.



**Fig. 2. Effect of leaf essential oil of *C. sinensis* on hepatic glucose of alloxan- induced diabetic rats**

Bars are expressed as mean of five replicates  $\pm$  S.E.M and error bars with different superscripts are statistically different ( $P = .05$ ), NC – Normal control; DC – Diabetic control; DR – Diabetic treated with reference drug; DO – Diabetic treated with leaf essential oil of *Citrus sinensis*



**Fig. 3. Effect of leaf essential Oil of *C. sinensis* on hepatic glycogen of alloxan- induced diabetic rats**

Bars are expressed as mean of five replicates  $\pm$  S.E.M and error bars with different superscripts are statistically different ( $P = .05$ ), NC – Normal control; DC – Diabetic control; DR – Diabetic treated with reference drug; DO – Diabetic treated with leaf essential oil of *Citrus sinensis*

## 3.2 Discussion

### 3.2.1 Chemical composition of leaf essential oil of *C. sinensis*

The major chemical constituents of the *Citrus sinensis* leaf essential oil identified in this study include monoterpenes such as  $\beta$ -phylendrene (22.85%), 3-Carene (12.45%), D-Limonene (4.96%), Caryophyllene (2.15%) and  $\beta$ -Pinene (1.39%). This finding is similar to the findings of [9] who studied the volatile components and antimicrobial activity of *C. sinensis* var. *mosammi* leaf oil.

### 3.2.2 Fasting blood glucose concentration

Type I diabetes is characterized by a loss of insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. In this study, administration of alloxan to the experimental animals led to the destruction of beta cells, a result that is consistent with several studies in rats [19-22]. Findings of this study clearly indicated that diabetic animals treated with leaf essential oil of *Citrus sinensis* had a reduced blood glucose concentration when compared to the diabetic control animals, a result similar to what was obtained with the diabetic animals treated with reference drug (metformin) (Fig. 1). The hypoglycemic effect observed may be attributed to the presence of monoterpenes in the essential oil. The hypoglycemic effect of monoterpenes, which are the major compounds identified in the *studied plant*, was previously demonstrated [23]. Monoterpenes may have insulin-mimetic properties or may be able to induce insulin production from the surviving beta cells enough to facilitate glucose uptake from the blood.

### 3.2.3 Hepatic glucose and glycogen

Alloxan destroys the insulin-producing beta-cells, consequently leading to an inhibition of glycogenesis with a simultaneous induction of glycogenolysis. In this study, hepatic glucose concentration was raised in the diabetic animals, which signifies increased production of glucose in the liver as a result of excessive glycogenolysis. However, administration of the oil caused a significant reduction in hepatic glucose concentration.

Glycogen concentration is directly proportional to insulin level [24]. Insulin promotes intracellular glycogen deposition by stimulating glycogen synthesis and inhibiting glycogen phosphorylase [25]. Hepatic glycogen was significantly reduced in this study beyond control level. This is expected following intraperitoneal injection of alloxan which compromised insulin production and by implication glycogen metabolism. Administration of the oil however led to a reversal of this feat. This action may be attributed to the ability of the constituents of the essential oil (especially the monoterpenes) to cause a restoration to normal of glycogen metabolism.

## 4. CONCLUSION

This study clearly shows that the leaf essential oil of *Citrus sinensis* has shown a significant antihyperglycemic effect which may be attributed to the presence of monoterpenes which has been reported in literature to possess antidiabetic property. Monoterpenes may elicit these effects either by mimicking insulin, regenerating dead beta cells or inducing production of insulin by surviving beta cells.



## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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