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Antidiabetic Activity of Phenolic Compounds from *Dregea volubilis* [Benth] Leaves in Streptozotocin Induced Diabetic Rats

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Abstract: The objective of the present study was to evaluate the effect of active compounds from *Dregea volubilis* [Benth] leaves on serum glucose in normal and diabetic rats. Diabetes was induced by Streptozotocin (STZ) and High Fat Diet (HFD) in wistar rats. An isolated fraction of ethanolic extracts of *Dregea volubilis* [Benth] (ETDV) was administered orally at a dose of 100 mg/kg, p.o. Metformin was used as standard anti diabetic drug (50 mg/kg, p.o). An isolated fractions showing for higher anti diabetic activity was subjected to column chromatography that led to isolation of a pure compound, which was given trivial name DV-1. The interesting results of our preliminary studies with the ETDV have motivated to isolate anti-diabetic active compounds from the leaves of DV for the management of hypoglycaemic activities. Collected fractions were subjected to anti-diabetic activity in STZ and HFD induced wistar rats. The fraction F from ETDV showed strong anti-diabetic activity on a par with the standard drug metformin. To ensure the compounds responsible for anti-diabetic activities associated with F respectively. In addition a column chromatographic analysis was carried out with F using various solvent systems and isolated compound named as DV-1 from the column which was amorphous powders with decomposition point. DV-1 is phenolic compound nature confirmed by spectral analysis. Reduction in the FBG by Dv-1 indicates that Dv-1 has anti diabetic efficacy and provides a scientific rationale for the use as an anti diabetic agent.

Key words: Blood Glucose · *Dregea volubilis* · Phenolic Compounds

INTRODUCTION

The chemistry of natural products is an emerging location in drug improvement activity. The secondary metabolite derived from plant and animal resources are proved to be a powerful therapeutic agent in various illnesses [1]. Naturally the secondary metabolites of the plant provide defence mechanisms towards predators, pathogens and for self protection towards herbivory and microbes [2]. The scientists are exploiting the natural merchandise of the plant and they may be focusing their attention to isolate the secondary metabolites of the plant and animals for treating diverse illnesses [3]. The vital plant secondary metabolites are particularly alkaloids, glycosides, tannins, lignins, flavanoids, terpenes, unstable oils, fixed oils, steroids so on [4]. The chemistry of natural merchandise facilitates the scientists to find out the structure of the secondary metabolites by way of the use of various separation strategies such as Colum chromatography, thin layer chromatography (TLC) and complex analytical strategies which include UV, IR, NMR and Mass spectroscopy. currently, at the least 119 chemical materials derived from ninety plant species may be considered as critical drugs which are in use in a single or greater nations [5]. Among those a number of the a success drugs are remoted from the natural sources which include antibiotic "penicillin'' from *Penicillium notatum*, antimalarial agent "quinine'' from *Cinchona succirubra*, Narcotic analgesic aspirin precursor "salicin'' from white willow bark *Salix alba*, Cardiac glycoside "digoxin'' from *Digitalis purpurea* and so on Viktorin and Sartorius [6]. Recently, anticancer dealers "vincristine and vinblastine" are isolated from the *Catharanthus roseus*; and these agents are successfully prescribed by means of the

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physicians for the treatment of cancer [7]. The interesting results of our initial studies with the ethanolic extracts of *Dregea volubilis* [Benth] (ETDV) [8] have motivated to isolate anti-diabetic responsible compounds from the leaves of *Dregea volubilis* [Benth] for the management hypoglycemic and hypolipidemic activities.

MATERIALS AND METHODS

Preparation of Different Plant Extracts: *Dregea volubilis* [Benth] leaves had been accrued from the wooded area of kalakatu, Tirunelveli District, India. Taxonomic identity was crafted from botanical survey of medicinal Plants, Siddha Unit, Government of India, Palayamkottai, Aunthenticated by Chelladurai Botonist. A voucher specimen No (CCRAS-168/2011). Fresh plant leaves had been shade dried at room temperature, floor into nice powder and saved in airtight containers. Then extracted (amount 500 g) with solvents of increasing polarity consisting of petroleum ether, ethyl acetate and ethanol, for 72 hours with each solvent, by continuous hot extraction the use of the soxhlet apparatus at a temperature of 60°C. The extracts were concentrated below decreased stress using a rotary evaporator to steady weight. The extracts had been amassed and preserved in a desiccator until used for further research.

Fractionation, Isolation, Purification and Characterization of Compounds from the Ethanol Extract: Chromatographic techniques have been used for the isolation of compounds from the fractions. The column chromatographic technique most generally used for the separation of compounds into numerous fractions in step with the affinity or solvating capacity of the compounds to the solvent used. The observe involves in fractionation and isolation of compounds from pharmacologically active ethanol extract. The structure of the compound had been attempted to set up through spectroscopic techniques.

Study Design: In order to carry out column chromatography, a solvent gadget was mounted with the aid of growing TLC technique. The silica gel (100-200 mesh size) slurry was made with the solvent system installed in advance. The slurry became poured time to time into the column very carefully and the silica gel became allowed to settle all the way down to from a uniform packing. Then the stop-cock of the column changed into opened and the excess of solvent over the column head became allowed to run. The dry crude ethanol extract (10g) changed into blended with small amount of silica gel in a mortar to get a free flowing powder. The powdered sample changed into then implemented cautiously at the pinnacle of the prepared column and efficiently eluted with solvent/solvent system the usage of diverse solvent structures consisting of petroleum ether, petroleum ether: chloroform, chloroform: ethyl acetate, ethyl acetate, ethyl acetate: methanol and methanol alone to separate the eluate. The eluate with equal R_f value are pooled collectively and evaporated to dryness. When the mixture of solvent system used, the ratio of mixtures are prepared as 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. Elutes had been gathered in some of conical flasks marked from fractions 1-100. Elutes had been noticed efficiently on TLC Plate and the flasks having similar spots were mixed together.

Animals: Wistar rats each weighing 150-200 g was received from Annamalai University at Chidambaram, Tamil Nadu, India. The suggestions of the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the authorities of India had been observed and earlier permission became granted from the Institutional Animal Ethics Committee (No. 842/CPCSEA). Rodent laboratory chow and water have been accessed ad libitum and rats were maintained on a 12 h light/dark cycle in a temperature regulated room (20-25°C) for the duration of the experimental strategies

Effect of Ethanolic extract of *Dregea volubilis* (ETDV) on Fasting Blood Glucose in STZ Induced Diabetic Rats: Various isolated fractions of ETDV (100 mg/kg) were evaluated for their anti-diabetic effect in fed with high energy diet of 20% sucrose and 10% lard. The STZ was freshly dissolved in citrate buffer (0.01 mol/L, pH 4.5) and stored on ice earlier to apply. One week later STZ inductions of diabetes in wistar rats, the fasting blood glucose levels were measured. The hyperglycemic rats (blood glucose >240 mg/dl) were divided into 10 groups (each with 3 rats). Distilled water, metformin and numerous remoted fractions of ETDV (100 mg/kg) everyday administered orally to normal control, diabetic control and the treatment groups respectively for 3 weeks.

Purification of Isolated Fraction: Nearly 1 gm of the fraction is weighed and mixed with silica gel and poured into the column. The column is eluted with different solvents by polarity foundation. Aliphatic fractions are separated via the solvent petroleum ether. In this solvent gadget, few distinct bands are produced. That fraction is

gathered and evaporated to dryness. The aromatic fractions are separated by the n-hexane: ethyl acetate mixture in the ratio of 1:1. The polar fraction locate in the extract is eluted by usage of chloroform. The second polar fraction of the extract is eluted by usage of methanol. The column fraction which is gathered and evaporated to dryness is used for additional studies [9].

Analysis of Fraction: The fraction became characterized via spectroscopy strategies like Perkin-Elmer Vector 22 version FT-IR Spectrophotometer (Nujol),1H NMR spectra were recorded in a BRUKER DPX-200 MHz using TMS as internal standard and Mass spectrometer spectra became recorded in SHIMADZU QP 50000 [10].

Statistical Analysis: Data expressed as mean ± SEM. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison test using Instat-3 software package (Graph pad), Prism Ltd, USA.

RESULTS

Column Chromatography Study with ETDV: The column chromatography was carried out with ETDV to split the eluates namely F1-40 using petroleum ether as a solvent system. F 41-75 are the eluates remoted the usage of petroleum ether: chloroform, F 76-105 are the eluates remoted the usage of chloroform, F 106-140 are the eluates remoted the usage of chloroform: ethyl acetate, F 141-160 are the eluates remoted the usage of ethyl acetate, F 161-183 are the eluates remoted the usage of ethyl acetate: methanol and finally methanol alone is used the eluate F 184-200. The volume of the each eluate is 50 ml. The eluates with same R_f value were pooled collectively and evaporated to dryness. The pooled fraction of ETDV such as F 1-40, F 41-75, F 76-105, F 106-140, F 141-160, F 161-183 and F 184-200 are named as A, B, C, D, E, F and G respectively. The pooled eluates of A, B, C, D, E, F, G had been examined in fasting blood glucose level in STZ induced diabetic rats.

Effects of 3-week administration of various isolated fractions ETDV (100 mg/kg) and Metformin (50 mg/kg) on FBG in STZ induced diabetic Rats: From the study it was determined that the fraction "F" showed significant (P<0.05) reduce in blood glucose however the different fractions did not longer display widespread effect of blood glucose when in comparison with normal control. The results of the effect of various isolated fractions of ETDV (100 mg/kg) on the blood glucose level in STZ prompted diabetic rats are proven in Table 1.

Purification of Pooled Column Fraction of ETDV by Column Chromatography: From the consequences of anti-diabetic effect, the fraction F from ETDV showed promising results. Subsequently this fraction became subjected to further purify the usage of column chromatography and followed by using TLC. The natures of fractions acquired are indexed in Table 2.

Characterization of Compounds Using Various Analytical Techniques

IR Studies with DV-1: The IR spectra showed characteristic absorption bands at 1587.08 cm^{-1} which show that the compounds have -OH bending and C = O stretching is found to be at 1628.30 cm^{-1}. The IR spectra exhibits characteristics absorption bands at 2881.55 cm^{-1}, 2942 cm^{-1} which observes that the compound is aliphatic -CH stretching and 3087.87 cm^{-1} shows the aromatic -CH stretching and 3450.45 cm^{-1} show -OH stretching. The spectrum is given in Figure 1.

Table 1: Effect of Various Isolated Fractions of ETDV (100 mg/kg) on the Blood Glucose Level in STZ Induced Diabetic Rats

	Fasting blood glucose			
Treatment	0 day	7th day	14th day	21st day
Normal control	78.4±3.7	77.9±4.2	78.5±2.7	76.6±3.6
Diabetic control	67.3±5.8	261.8±5.3	259.3±4.8	251.4±2.8
Fraction-A	69.9±2.4	263.7±4.7	257.4±2.3	228.8±4.6
Fraction-B	75.6±4.1	259.5±3.9	251.3±3.4	217.4±3.5
Fraction-C	79.8±8.5	265.4±2.8	249.5±2.3	198.8±2.6
Fraction-D	65.3±4.6	245.3±3.5	218.4±4.6	181.1±5.3
Fraction-E	68.4±2.9	255.4±4.9	221.5±2.5	167.4±5.2
Fraction-F	76.5±7.9	248.3±7.1	150.4±5.8	85.5±4.6*
Fraction-G	77.4±7.6	257.4±6.5	185.3±4.6	149.3±5.2
Metformin	80.3±3.4	251.4±5.5	142.3±3.1	87.4±6.1*

n=3. * P < 0.05 *vs* control group

Table 2: The Column Chromatographic Fractions of F from ETDV and their TLC Analysis

S. No	Solvent system used in column elution	Eluates	Volume of pooled eluate (ml)	Solvent system used for TLC	Nature of compound
1.	Ethyl acetate: Hexane (25: 75)	F 1-F 5	300	Methanol: Ethyl acetate (20: 20)	
2.	Ethyl acetate: Hexane (50: 50)	F 6-F 10	300	Methanol: Ethyl acetate (20: 20)	
3.	Ethyl acetate: Hexane (75: 25)	F 11-F 19	300	Methanol: Ethyl acetate (20: 20)	
4.	Ethyl acetate alone (100)	F 20-F 25	300	Methanol: Ethyl acetate (20: 20)	
5.	Methanol: Ethyl acetate (5: 95)	F 26-F 31	300	Methanol: Ethyl acetate (20: 20)	
6.	Methanol: Ethyl acetate (10: 90)	F 32-F 41	300	Methanol: Ethyl acetate (20: 20)	
7.	Methanol: Ethyl acetate (20: 80)	F 42-F 45	300	Methanol: Ethyl acetate (20: 20)	
8.	Methanol: Ethyl acetate (50: 50)	F 46-F 47	300	Methanol: Ethyl acetate (20: 20)	Amorphous powder with
9.	Methanol alone (100)	F 48-F 50	100	Methanol: Ethyl acetate (20: 20)	decomposition point *

* is the compound from the fractions of (F 46 - 47) named as DV -1.

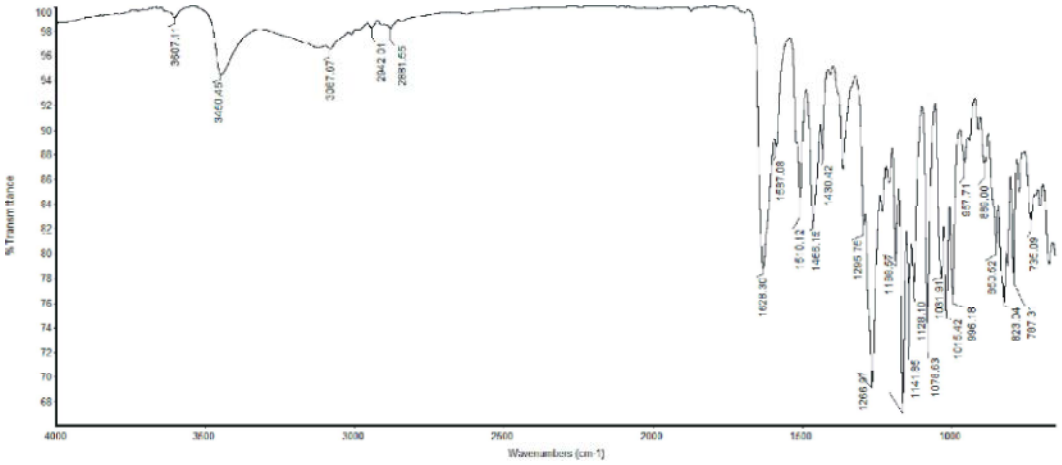

Fig. 1: IR Spectrum of the Compound DV-1 from ETDV

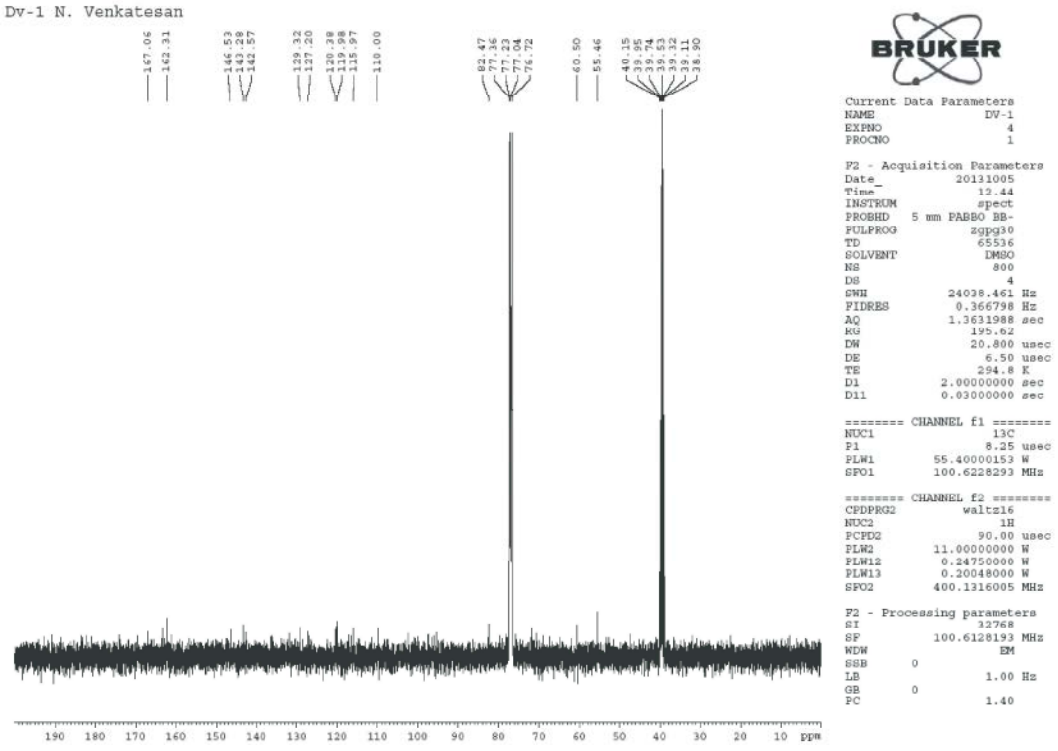

Fig. 2: ^{13}C-NMR Spectrum of DV-1 from ETDV

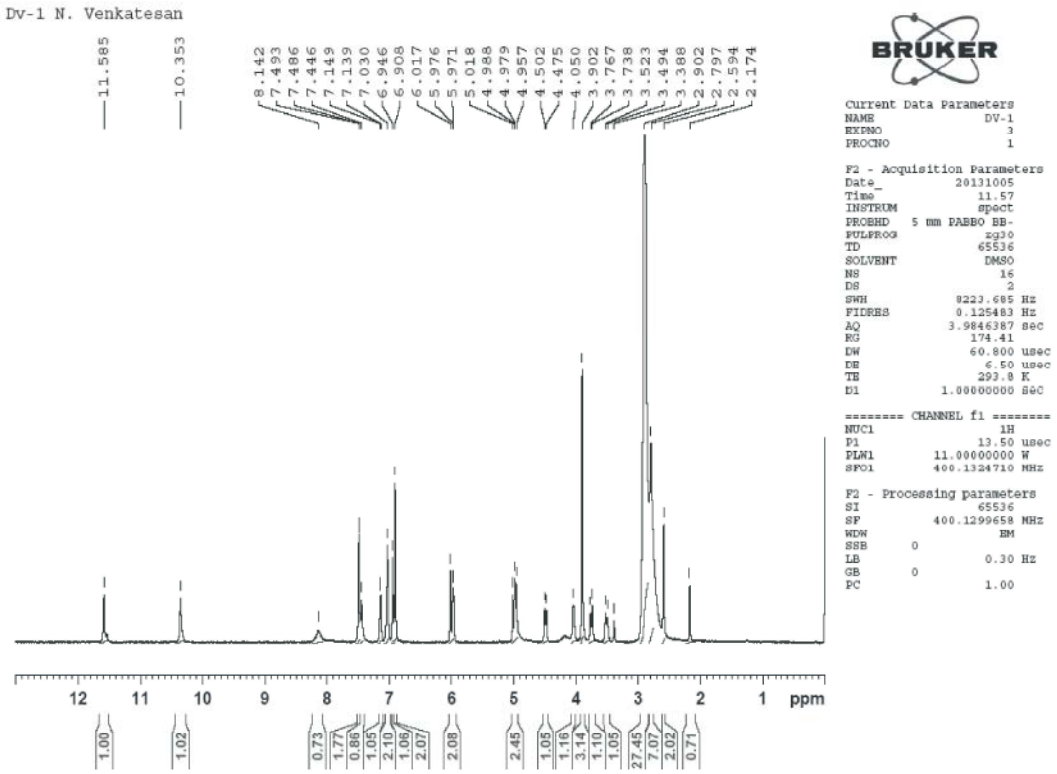

Fig. 3: ^{1}H - NMR Spectrum of DV-1 from ETDV

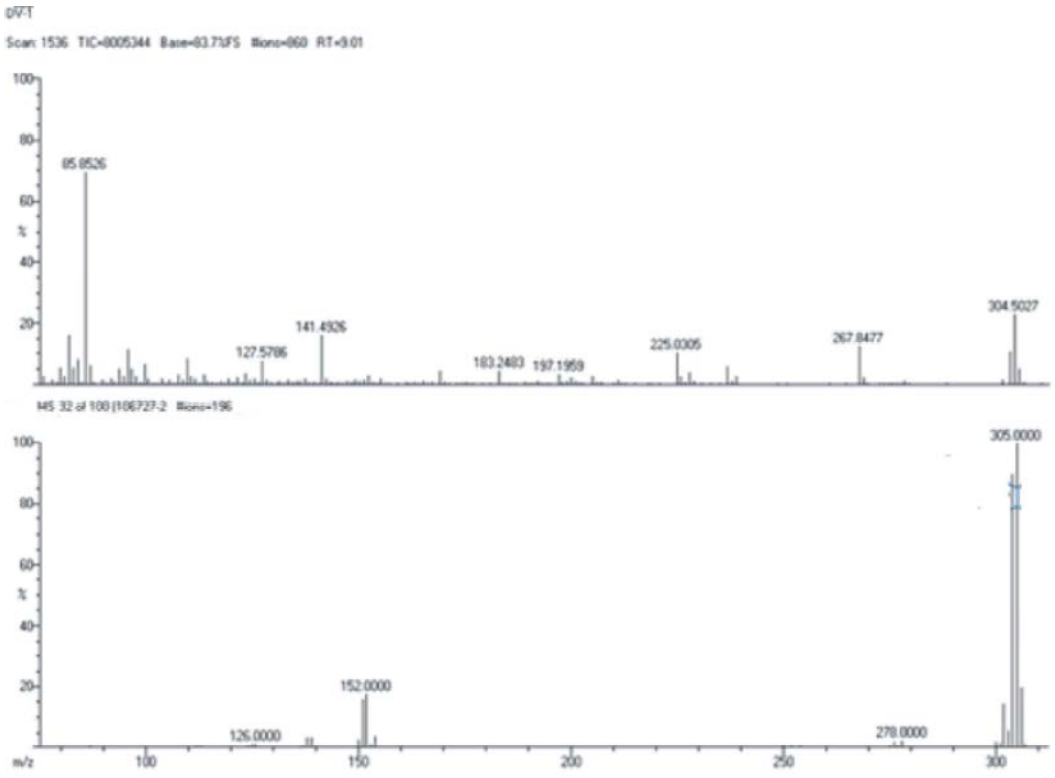

Fig. 4: Mass Spectrum of DV-1 from ETDV

^{13}C-NMR Studies with DV -1: From the spectra it was showed that the spectrum showed 25 signals. It revealed that the chemical shifts were at δ 55.46 ppm (-OCH_3), δ 60.50 ppm (-CH_2), δ 82.47 ppm (-C-OH), δ110.00 - 120.38 ppm (O-C), δ 127.20-146.53 ppm (Aromatic carbon) δ 162.31 ppm (-C=O). The spectrum of the compound is given in Figure-2.

^{1}H - NMR Studies with DV- 1: From the spectra it was reported that -CH_2 at δ_0.71_ppm and -OCH_3 at δ 3.14 ppm. Aliphatic -CH observed that δ 2.45, δ 2.08 ppm. Aromatic -CH observed that δ 2.07, δ 1.06, δ 2.10, δ 1.05, δ 0.88, δ 1.77 ppm. -OH resonated at δ 0.73, δ 1.02 and δ 1.00. The spectrum of the compound is presented in Figure-3.

Mass Spectrum Studies with DV-1: From the mass spectrum of DV - 1, it was observed that a molecular ion peak at signal *m/z* = 304.50. The observed fragmentation pattern shows that the similarity of a compound having an aromatic origin. The spectrum of the compound is given in Figure 4.

DISCUSSION

Now a day, the interest in the study of herbal product is growing rapidly, especially as a part of drug discovery packages. In our previous research proved that the anti-diabetic activities are associated with the active constituents of ETDV [11]. In continuation to the previous research, we have shown interest to isolate the pure ingredients accountable for the above mentioned pharmacological response. The initial study was achieved with GC-MS evaluation, the effects showed that there are fifteen compounds in ETDV [12]. A try was made to isolate the purified compounds responsible for anti-diabetic response the use of column chromatography method with ETDV. The fraction F from ETDV showed high anti-diabetic interest on a par with the standard drug metformin. To ensure the compound chargeable for anti-diabetic response associated with F respectively, In addition a column chromatographic analysis became finished with 'F' using different solvent systems. We isolated one compound named as DV-1 from the column which became amorphous powders with decomposition factor; but DV-1 is phenolic compound nature confirmed by using spectral evaluation. At gift, the exact Mechanism of action of phenolic compounds which help in the rejuvenation of the pancreatic beta cells in diabetic animals and restore their capacity to secrete more hormones.

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Author's Contributions: AS supervised the experimental design and laboratory analysis. VN carried out the experiments, analyzed the data and wrote the manuscript. BAV corrected the manuscript. All authors read and approved the final manuscript.

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