ISOLATION AND CHARACTERIZATION OF ANTI-DIABETIC COMPOUND FROM D*REGEA VOLUBILS* [BENTH.] LEAF

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Abstract— The available drugs for diabetes, Insulin or Oral hypoglycemic agents have one or more side effects. Search for new antidiabetic drugs with minimal or no side effects from medicinal plants is a challenge according to WHO recommendations. In this aspect, the present study was undertaken to evaluate the effect of active compounds from *Dregeavolubilis* [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 *Dregeavolubilis* [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.

Keywords— Blood glucose, *Dregeavolubilis*, Phenolic compound.

I. INTRODUCTION

The chemistry of natural products is an emerging area in drug development activity. The secondary metabolite derived from plant and animal sources are proved to be an effective therapeutic agent in various diseases [1]. Naturally the secondary metaboilites of the plant provide defence mechanisms against predators, pathogens, and for self protection against herbivory and microbes [2]. The chemistry of natural products helps the scientists to find out the structure of the secondary metabolites by using various separation techniques such as Colum chromatography, thin layer chromatography (TLC) and sophisticated analytical techniques such as UV, IR, NMR and Mass spectroscopy. Currently, at least 119 chemical substances derived from 90 plant species can be considered as important drugs that are in use in one or more countries [3]. The interesting results of our preliminary studies with the ethanolic extracts of *Dregeavolubilis* [Benth] (ETDV) [4] have motivated to isolate anti-diabetic active compounds from the leaves of DV for the management hypoglycaemic and hypolipidemic activities.

II. MATERIALS AND METHODS

Dregeavolubilis [Benth] leaves were collected from the forest of kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottaiaunthenticated by ChelladuraiBotonist, voucher specimen No (CCRAS-167/2011). Fresh plant leaves were shade dried at room temperature, ground into fine powder and stored in airtight containers. Then extracted (amount 500 g) with solvents of increasing polarity such as petroleum ether, ethyl acetate, and ethanol, for 72 hours with each solvent, by continuous hot extraction using the soxhlet apparatus at a temperature of 60°C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

Chromatographic techniques were used for the isolation of compounds from the fractions. The column chromatographic technique most commonly used for the separation of compounds into several fractions according to the affinity or solvating capacity of the compounds to the solvent used. The study involves in fractionation and isolation of compounds from pharmacologically active ethanol extract. The structure of the compound were tried to establish by spectroscopic methods.

In order to carry out column chromatography, a solvent system was established by developing TLC technique. The silica gel (100-200 mesh size) slurry was made with the solvent system established earlier. The slurry was poured time to time into the column very carefully and the silica gel was allowed to settle down to from a uniform packing. Then the stop-cock of the column was opened and the excess of solvent over the column head was allowed to run. The dry crude ethanol extract (10 g) was mixed with small amount of silica gel in a mortar to get a free flowing powder. The powdered sample was then applied

carefully on the top of the prepared column and successfully eluted with solvent/solvent system using various solvent systems such as petroleum ether, petroleum ether: chloroform, chloroform: ethyl acetate, ethyl acetate, ethyl acetate: methanol and methanol alone to separate the eluate. The eluate with same Rf value are pooled together 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 were collected in a number of conical flasks marked from fractions 1-100. Elutes were spotted successfully on TLC plate and the flasks having similar spots were combined together.

Male Wistar rats each weighing 180–220 g was obtained from RMMCH in Annamalai University at Chidambaram, Tamil Nadu, India. The guidelines of the Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA) of the Government of India were followed, and prior permission was granted from the Institutional Animal Ethics Committee (No. 842/CPCSEA). Rodent laboratory chow and water were accessed ad libitum, and rats were maintained on a 12 h light/dark cycle in a temperature regulated room (20–25 °C) during the experimental procedures.

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 kept on ice prior to use. One week later STZ inductions of diabetes in wistar rats, the fasting blood glucose levels were measured [5]. The hyperglycemic rats (blood glucose >240 mg/dl) were divided into 10 groups (each with 3 rats). Distilled water, metformin and various isolated fractions of ETDV (100 mg/kg) daily administered orally to normal control, diabetic control and the treatment groups respectively for 3 weeks.

The fraction was characterized by spectroscopy techniques like Perkin-Elmer Vector 22 model FT-IR Spectrophotometer (Nujol),1H NMR spectra were recorded in a Bruker DPX-200 MHz using TMS as internal standard and Mass spectrometer spectra was recorded in Shimadzu QP 50000 and was given a trivial name DV - 1.

III. RESULTS AND DISCUSSION

The column chromatography study was carried out with ETDV to separate the eluates namely F 1 – 40 using petroleum ether as a solvent system. F 41 – 75 are the eluates isolated using petroleum ether: chloroform, F 76 – 105 are the eluates isolated using chloroform, F 106 – 140 are the eluates isolated using chloroform: ethyl acetate, F 141 – 160 are the eluates isolated using ethyl acetate, F 161 – 183 are the eluates isolated using 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 together and evaporated to dryness. The pooled fraction of DVET 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 were tested in fasting blood glucose level in STZ induced diabetic rats.

From the study it was observed that the fraction "F" showed significant (P<0.05) decrease in blood glucose but the other fractions did not show significant effect of blood glucose when compared with normal control. The results of the effect of various isolated fractions of ETDV (100 mg/kg) on the blood glucose level in STZ induced diabetic rats are shown in Table 1.

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

Treatment	Fasting blood glucose			
	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

From the results of anti-diabetic effect, the fraction F from ETDV showed promising results. Hence this fraction was subjected to further purify using column chromatography and followed by TLC.

The IR spectra exhibit characteristic absorption bands at 1587.08 cm^{-1} which show that the compound have -OH bending and C = O strecthing 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 shows 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 presented in Figure 1.

From the spectra it was observed that the spectrum showed 25 signals. It revealed that the chemical shifts were atδ 55.46 ppm (-OCH_3), δ 60.50 ppm (-CH_2), δ 82.47 ppm (–C–OH), δ 110.00 - 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.

From the spectra it was observed that –CH_2 at δ 0.71 ppm and –OCH_3 at δ 3.14 ppm. Aliphatic –CH observed thatδ 2.45, δ 2.08 ppm. Aromatic –CH observed thatδ 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 andδ 1.00.

The spectrum of the compound is given in Figure 3. 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 Figure4.

Now a day, the interest in the study of natural product is growing rapidly, especially as a part of drug discovery programs. In our previous studies proved that the anti-diabetic activities are associated with the active constituents of ETDV [4]. In continuation to the previous study, we have shown interest to isolate the pure constituents responsible for the above mentioned pharmacological action. The initial study was carried out with GC – MS analysis, the results showed that there are fifteen compounds in ETDV [6]. An attempt was made to isolate the purified compounds responsible for anti-diabetic activity using column chromatography technique with ETDV. The fraction F from ETDV showed strong anti-diabetic activity on a par with the standard drug metformin. To ensure the compound responsible for anti-diabetic activities associated with F respectively, In addition a column chromatographic analysis was carried out with 'F' using various solvent systems. We isolated one compound named as DV-1 from the column which was amorphous powders with decomposition point; however DV-1 is phenolic compound nature confirmed by spectral analysis. At present, the exact Mechanism of action of the isolated compound of DV - 1 is not yet known and will be the subject of further studies.

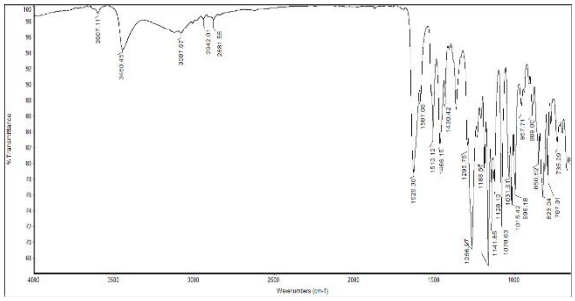

Fig. 1. IR Spectrum of the Compound DV-1 from ETDV

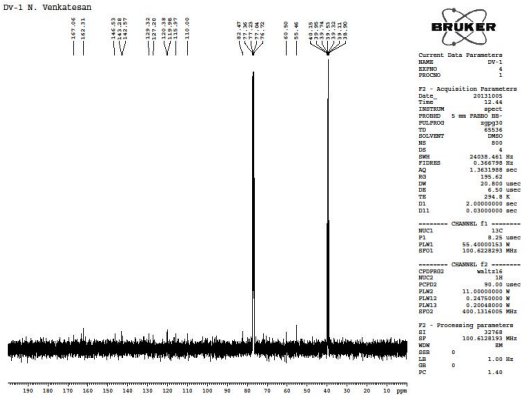

Fig. 2.^{13}C-NMR Spectrum of DV – 1 from ETDV

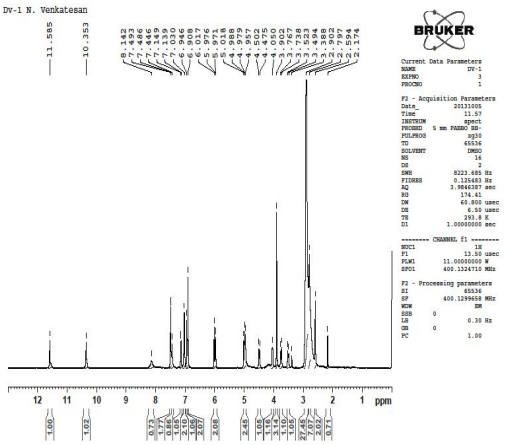

Fig. 3.^{1}H - NMR Spectrum of DV – 1 from ETDV

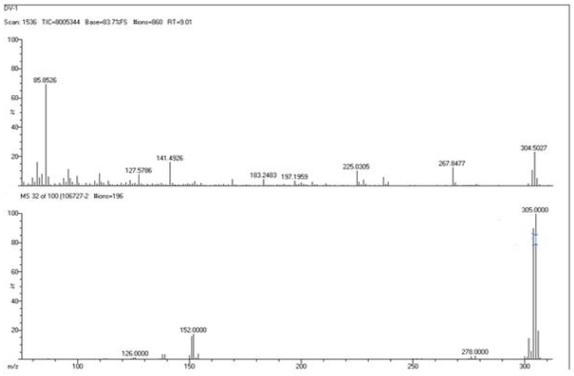

Fig. 4. Mass Spectrum of DV – 1 from ETDV

CONCLUSIONS

The isolated compound from 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

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