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Evaluation of the hypoglycaemic activity of *Bauhinia monandra* leaf in Alloxan- diabetic rats and INS-1 insulin cells

G.O Alade[1], O.R. Omobuwajo[1*], C.A. Adebajo [2] and E.J. Verspohl[3]

[1] *Department of Pharmacognosy & Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria*
[2] *Department of Pharmacognosy, Faculty of Pharmacy,Obafemi Awolowo University, Ile-Ife, Nigeria*
[3]*Department of Pharmacology, Institute of Pharmaceutical and Medicinal Chemistry, Westfalische Wilhelms University, Hittorfstraße 58 - 62, Münster, Germany*

ABSTRACT

The plant, Bauhinia monandra Kurz, (Caesalpinaceae) is an ornamental. Traditionally, the leaves are used in the treatment of diabetes. 4.150 kg of the dried leaves of B. monandra was extracted with methanol (cold extraction), concentrated in vacuo to obtain 300 g of extract. Oral administration (p.o.) of the methanolic extract at 2 g/kg administered to alloxan-diabetic rats showed significant blood glucose reduction of 65% at the end of a 4 hour period similar to the effect of glibenclamide (5 mg/kg, p.o). The methanolic extract was successively partitioned into ethylacetate, butanol and water fractions, and the same test showed that the butanol fraction (2 g/kg) had 67.4 %, Water fraction had 71.5 % hypoglycaemic activity at 4 hours after oral administration comparable to that of glibenclamide (67.8 %) in the in vivo model. Subfractions of the butanol fraction (BMBuF7; 1 g/kg, BMBuF7C; 0.75 g/kg) reduced hyperglycaemia in alloxan-diabetic rats to 62 and 66 % respectively and induced insulin release from INS-1 cells. Quercetin-3-rutinoside was isolated from the butanol fraction and characterrized. The results justify the ethnomedical use of the plant in the management of diabetes and sugests that stimulating insulin release is one of the modes of action of the butanol fraction and some of its subfractions.

Keywords *Bauhinia monandra*, hypoglycaemic activity, Quercetin, blood glucose.

INTRODUCTION

Diabetes mellitus, a disease involving chronic metabolic disorders of carbohydrate, protein and fat due to relative or absolute lack of insulin and various degrees of insulin resistance [1,2,3,]. In

the year 2000, the estimated occurrence of diabetes in Nigeria was 1.707 million. It is projected that by 2030, the number of diabetes would be 4.835 million [4]. The list of drugs available for management of diabetes is short and more drugs are still needed. Thererfore, the discovery of more drugs which may have new modes of action is very pertinent [5]. Traditional plant remedies have always provided sources of useful hypoglycaemic agents [6,7,8,9,10] and therefore, should continue to be investigated for possible drug alternatives.

Bauhinia monandra Kurz, (Caesalpinaceae) is native of Burma and a fast growing tree or shrub commonly cultivated as an ornamental plant in the Tropics [11]. Folklorically, it is used for the treatment of diabetes in Brazil [12]. Lectins, β-carotene, fatty acids such as myristic, oleic, linolenic and linoleic acids, calcium, phosphorus and potassium have been reported from the leaf [13,14,15]. Two flavonoids; quercetin and quercetin-3-rutinoside were isolated from the leaves [16]. The only pharmacological report on the antidiabetic activity of the plant was carried out on the stem-bark [17], although there have been some reports for related species like *B. forficata, B. megalandra, B. candicans and B. cheilandra* [18,19,20,21,22,23,24,25]. There has been no investigation of the antidiabetic activity of the leaves even though it is commonly used locally [A.T. Oladele, Obafemi Awolowo University, Ile-Ife, Nigeria, Personal communication]. Therefore, we were interested in investigating the antidiabetic effect of the leaf of *B. monandra* on alloxan-diabetic rats and on INS-1 insulin cells.

EXPERIMENTAL SECTION

Materials

Alloxan-monohydrate and glibbenclamide were from Sigma-Aldrich, 3050 Spruce St, St. Louis, MO 63103, USA; Glucotrend^R 2 glucometer and Lancet with Auparavant glucotrend^R 2 strips from Roche Diagnostic, GmbH, Mannheim, Germany were used for the hypoglycaemic testing. Halothane was from Fluka Chemie GmbH, Steinheim, Germany. Rat insulin was from Novo Nordisk, Bagsvaerd, Denmark and (mono mono ^{125}I – Tyr A 14) porcine insulin was from Aventis, Frankfurt, Germany, and anti-insulin antibodies were from Linco, St. Louis, USA.

INS-1 cells

Dr. C.B. Wollheim (University of Geneva, Geneva, Switzerland) generously provided INS-1 cells, an insulin releasing insulinoma cell line. Biological chemicals/media used for the INS-1 cell experiment were obtained as previously reported [26,27]. The *in vitro* tests were carried out at the Institute of Pharmaceutical and Medicinal Chemistry, Munster, Germany.

Animals

Healthy male Wistar rats weighing 175±25 g bred under standard conditions [temp. 27±3^o C, relative humidity 65 %] at the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria under natural 12 h daylight/ night conditions. They were fed on a standard pellet diet [Bendel Feeds, Nigeria] and given free acess to water. Guide for the care and use of Laboratory Animals were followed in this study [28].

Plant material

The leaves of *Bauhinia monandra* Kurz were collected in Obafemi Awolowo University (OAU), Ile-Ife, Nigeria in December 2002 and authenticated by Mr. B.O. Daramola, [Taxonomist]

Department of Botany, OAU, Ile-Ife. A voucher specimen FHI 106762 was deposited in the herbarium of Forestry Research Institute of Nigeria, Ibadan, Nigeria.

Instrumentation

Silica gel 60HR (Merck, TLC grade without binder) was used for vacuum liquid chromatography (VLC). Pre-coated silica gel plates, (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for analytical thin layer chromatography (TLC). Diaion^R HP20 was from Merck, Germany. A Merck semi-preparative HPLC, Eurospher column, 100-C_{18} was used. All NMR spectra were from a Bruker DRX 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C; solvent CD_3OD). 2D ^{1}H- ^{1}H-homocosy, HMBC and HMQC heterocosy were obtained from the same instrument.

Chromatography

System 1: For analytical thin layer chromatography (TLC), the following solvent systems were used; $CHCl_3$: MeOH : H_2O 7:3:0.2; $CHCl_3$: MeOH : AcOH 8:2:0.2, $CHCl_3$: MeOH : AcOH 7:3:0.5

System 2: The presence of the compound in the butanol subfractions were monitored on a HPLC Nucleodur column, 100-C_{18} 5 µm, dimension: 125 x 4.6 mm (Macherey-Nagal, Duren, Germany) eluted with gradient mixtures of (HPLC 1 method) MeOH-H_2O (5 % MeOH at 0 min; 30 % MeOH at 30 min.; 100 % MeOH at 45 min. Held up to 55 min., 5 % MeOH at 56 min. Held up to 65 min.) and (HPLC 2 method) MeOH-H_2O (30 % MeOH at 0 min; 70 % MeOH at 30 min.; 100 % MeOH at 45 min. Held up to 55 min., 30 % MeOH at 56 min. Held up to 65 min.), oven temperature: 25^{o}C, detector: UV-DAD MD 2010 (Jasco, Groβ-Umstadt, Germany): 200 – 450 nm, injection volume: 10 – 100 µL and flow rate: 1.4 mL/min.

System 3: For the isolation of the constituents a Eurosphere column with 10 µm, dimension 300 x 8 mm including HPLC grade solvents was used. Their purity was monitored on a HPLC Eurosphere column, 100-C_{18}, 5 µm, dimension: 125 x 4 mm (Dionex, Germany).

Plant extraction and Fractionation

Methanol extract: Air dried and powdered leaves (4.15 kg) were extracted with methanol (3 x 4500 Ml, 24 h each) to give a methanolic extract (BMMet, 300 g, 7.23 % w/w).

Partition fractions of the methanolic extract: The methanolic extract (BMMet, 290 g) was suspended in water and successively partitioned with and concentrated *in vacuo* to give ethylacetate (BMEt, 132.2 g), n-butanol (BMBu, 92.1 g), and aqueous (BMAq, 61.6 g) fractions with drug/extract ratios 45.6, 31.8 and 21.2 % w/w, respectively.

Chromatographic fractionation of the Butanol fraction: 85 g of the hypoglycaemic active n-butanol fraction BMBu was subjected to Vacuum Liquid Chromatography (VLC), (column dimension: 9 x 12, silica gel HR60,) eluted with gradient mixtures of $CHCl_3$, MeOH and H_2O. A total of 33 fractions of 250 ml each were collected and bulked into nine fractions, BMBuF1-9, BMBuF1 ($CHCl_3$ 100%; $CHCl_3$: MeOH 9:1, 500 ml, 310 mg), BMBuF2 ($CHCl_3$: MeOH 9:1, 1000 ml, 1.09 g), BMBuF3 ($CHCl_3$: MeOH 7:3, 500 ml, 1.45 g), BMBuF4 ($CHCl_3$: MeOH 7:3, 500 ml, 1.73 g), BMBuF5 ($CHCl_3$: MeOH 7:3; 5:5, 750 ml, 3.01 g), BMBuF6 ($CHCl_3$: MeOH 5:5; 3:7, 2750 ml, 27.24 g), BMBuF7 ($CHCl_3$: MeOH 3:7; $CHCl_3$: MeOH : H_2O 6:4:1; 5:5:1;

5:5:2, 1750 ml, 17.4 g), BMBuF8 ($CHCl_3$: MeOH : H_2O 4:6:4, 750 ml, 10.13 g, BMBuF9 (MeOH : H_2O 5:5, 250 ml, 2.83 g), after TLC ($CHCl_3$: MeOH : H_2O 7:3:0.2; $CHCl_3$: MeOH : AcOH 8:2:0.2, $CHCl_3$: MeOH : AcOH 7:3:0.5; BAW 4:1:5).

Chromatographic fractionation of the most active BMBuF7 and subfractions of the Butanol fraction:

12 g of BMBuF7 was similarly subjected to VLC (column dimension: 6 x 12 cm, silica gel HR60) eluted with gradient mixtures of $CHCl_3$, EtOAc, MeOH and H_2O . A total of 47 fractions of 150 ml were collected and bulked into eight fractions, BMBuF7A-H, BMBuF7A ($CHCl_3$ 100%; $CHCl_3$: EtOAc : MeOH 5:3.5:1.5; 900 ml, 126 mg), BMBuF7B ($CHCl_3$: EtOAc : MeOH 5:3.5:1.5; 4.5:3:2.5, 600 ml, 390 mg), BMBuF7C ($CHCl_3$: EtOAc : MeOH 4.5:3:2.5, 750 ml, 2.54 g), BMBuF7D ($CHCl_3$: EtOAc : MeOH 4:3:3, 1500 ml, 6.37 g), BMBuF7E ($CHCl_3$: EtOAc : MeOH 3:3:4; 1:3:6; 750 ml, 1.5 g), BMBuF7F ($CHCl_3$: EtOAc : MeOH 1:3:6, 300 ml, 1.6 g), BMBuF7G ($CHCl_3$: EtOAc : MeOH 1:3:6, 450 ml, 879 mg), BMBuF7H ($CHCl_3$: EtOAc : MeOH 1:3:6; EtOAc : MeOH 5:5; MeOH 100%; MeOH : H_2O 9:1 1800 ml, 498 mg) after TLC ($CHCl_3$: MeOH : AcOH 7:3:0.5; BAW 4:1:5) control.

Isolation of Constituents

Butanol fraction (BMBu, 2g) was subjected to a column chromatography (60 x 4 cm) over Diaion^R HP20, using 5 % increases in MeOH till 100 %. The first 13 fractions (150 mL) each were collected; monitored using Dionex HPLC (linear gradient of diluted aqueous ortho-phosphoric acid (Ph 2.0) to MeOH in 45 min, injection volume 20 µL, flow rate 1 ml/min) coupled to UV-Vis detector (200 – 595 nm). Fractions having similar UV profiles were bulked to give fraction **BMBu1** (1.08 g), **2** (40 mg), **3** (81 mg), **4** (94 mg), **5 - 10** (54 mg), **11** (13 mg), **12** (52 mg) and **13** (37 mg). Fractions **5 – 10** gave a 16 mg precipitate which was collected by filtration. The 38 mg of **BMBu5 - 10** was further purified on a semi preparative HPLC, eluted with gradient mixtures of MeOH-H_2O ((10% MeOH, 5 min; 100% MeOH, 35 min; 100% MeOH, 40 min and held up to 46 min.; 1 ml/min) to obtain more of the precipitate identified by spectroscopic (nmr, HMBC, LC MS) data as quercetin-3- rutinoside.

***In vivo* Experiments**

Each group of rats consisted of five rats. A group of normal rats fasted for eighteen hours and treated with 0.9 % (w/v) saline (0.5 ml vehicle) using an intragastric tube made up the group I (normoglycaemic control, NGR). Other rats were made diabetic by injecting alloxan-monohydrate (160 mg/kg body weight, i.p.) dissolved in 0.9 % saline and kept in the cages for 3 – 5 days (the rats were monitored 12 hourly for hyperglycaemia). Only the alloxan – induced hyperglycaemic rats with blood glucose levels higher than 11.1 mmol/L (200mg/dL) were selected and divided into 3 groups (II - IV). Group II consisted of rats given only saline (0.5 ml) being diabetic and served as negative control (DNC)while Group III consisted of diabetic rats given the drug to be tested (test group). Group IV contained rats getting 5 mg/kg of glibbenclamide in the vehicle (diabetic positive control, GLB). The initial blood glucose level of all the rats in groups I – IV at 0 h (T_0) and also at 1, 2 and 4 h (T_t) after treating with saline or drug using intragastric tube was determined by collecting blood samples from the venous pool of the tail vein after pricking the tail of the halothane-anaesthetised rats with a sterile lancet. The blood glucose level was determoined using a glucometer [29,30,31].

The extract/fractions of *B. monandra* prepared as fine suspension in 10% (v/v) NaCl were orally administered to the rats of group III at the doses of 0.5, 1.0, and 2.0 g/kg using intragastric tube. Glibenclamide (5 mg/kg) was similarly administered to group IV rats. The methanolic extract, its fractions (ethyl acetate, butanol and aqueous (dose; 2 g/kg), subfractions (BMBuF2 – 9) of the butanol fraction (BMBu) (dose; 1 g/kg), subfractions (BMBuF7C – F) of the butanol subfraction (BMBuF7) (dose; 0.75 g/kg) were investigated. Fractions BMBuF1, BMBuF7A – B and G – H were not investigated *in vivo* due to low weight. Blood was collected from the tail of the rats by pricking with a sterile Roche Glucotrend lancet at 0, 1, 2 and 4 hours after oral administration of the drug/extract/fraction. Glycaemic levels were determined in all the groups of animals using a glucometer [29,30,31].

Culture of INSI-1 cells and insulin radioimmunoassay

The INS-1 cells were grown in 24 multi-wells for 5-6 days (half confluence: 1 - 2 x 10^6 cells/ml) in RPMI medium supplemented with 10% (v/v) foetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Prior to the experiment, INS-1 cells were washed three times and then incubated with Krebs–Ringer buffer containing 10 mmol/L HEPES and 0.5% bovine serum albumin (KRBH) and 5.6 mM glucose for 90 minutes [26]. Each extract/fraction was checked for non-interference with the insulin radioimmunoassay. Half confluent cells in multi-wells were incubated for 90 minutes at 37^{o}C in Krebs-Ringer buffer containing HEPES and 0.5 % bovine serum albumin. Insulin released into the medium was determined by a radioimmunoassay using rat insulin as standard, (mono ^{125}I – Tyr A 14) porcine insulin as the labelled compound and anti-insulin antibodies [26]. Each extract/compound had been checked for non-interference with the insulin radioimmunoassay [26,27]. The value of insulin release induced by 5.6 mmol/L glucose was taken as 100 % (control) and the results of other test agents were expressed as percentage of this value. Data are not given as absolute amounts of secreted insulin since cultured cells are not identically grown from week to week at a distinct time point after the passage. The extracts/fractions and glibbenclamide (positive control) were tested at 5.6 Mm glucose. The insulin secretion stimulated by 3.0 mmol/L glucose was taken as a sub-stimulatory concentration (control experiment).

***In vitro* insulin release**

The ability of extracts and fractions (BMEt, BMBu and BMAq) to induce insulin release from INS-1 cells was determined at 0.01 and 0.1 mg/ml concentrations. Glibbenclamide (0.001 mg/ml) was used as a control. The compound isolated from the butanol fraction, the subfractions BMBuF1-9 and BMBuF7A – H were also tested at these concentrations.

Statistical analysis

Blood glucose levels were observed at 0 h (T_0) and 1, 2, 4 h (T_t) for the rats that received saline (negative control), extract/fraction (test) and glibenclamide (positive control). The results of the glucose lowering effect were calculated as the percentage decrease from initial value using the formula $\{T_t - T_0\}/T_0$ x 100. Data were expressed as the mean ± SEM. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Hypoglycaemic Activities of *Bauhinia monandra* leaf methanolic extract and its partitioned fractions

Table 1: Time Course of Blood Glucose changes in Alloxan Diabetic Rats administered *Bauhinia monandra* leaf methanolic extract and its Ethyl acetate, Butanol and Water fractions

Category of animal	Blood glucose level (mg/dl)			
	0HR	1HR	2HR	4HR
Normoglycaemic control	84 ± 0.6	86 ± 0.6 (-2.4%)	83 ± 0.5 (1.2%)	84 ± 0.6 (0.0%)
Diabetic negative control	277 ± 1.9	279 ± 1.9 (-0.7%)	277 ± 1.8 (0.0%)	281 ± 1.9 (-1.4%)
Methanol extract (BMMet, 0.5 g/kg)	295 ± 6.7	334 ± 5.0 (-13.2%)	328 ± 5.3 (-11.2%)	277 ± 6.5 (6.1%)
Methanol extract (BMMet, 1.0 g/kg)	450 ± 4.2	390 ± 7.6 (13.3%)	305 ± 5.8 (32.2%)	270 ± 5.7 (40.0%)
Methanolic extract (BMMet, 2.0 g/kg)	275 ± 1.9	235 ± 1.6 (14.5%)	113 ± 1.4 (59.0%*)	99 ± 0.9 (64.0%*)
Ethylacetate fraction (BMEt, 2.0 g/kg)	386 ± 3.8	404 ± 3.8 (-4.7%)	263 ± 4.0 (31.9%)	219 ± 3.7 (43.3%*)
Butanol fraction (BMBu, 2.0 g/kg)	402 ± 3.1	355 ± 3.5 (11.7%)	249 ± 1.7 (38.1%)	131 ± 1.5 (67.4%*)
Aqueous fraction (BMAq, 2.0 g/kg)	379 ± 2.2	305 ± 2.0 (19.5%)	178 ± 1.0 (53.0%*)	108 ± 1.0 (71.5%*)
Glibenclamide 5 mg/kg	341 ± 1.8	258 ± 2.0 (24.4%)	148 ± 0.4 (56.6%*)	109 ± 0.7 (67.8%*)

Values are statistically significant at $p < 0.05$; Percentages of blood glucose reduction relative to 0 hour are given in parenthesis; BMEt, BMBu, BMAq : partitioned fractions of BMMet extract of B. monandra leaf .*

Bauhinia monandra has been used folklorically for treating Diabetes mellitus. The hypoglycaemic activity of the stem bark on alloxan diabetic rats has been reported in which 1 g/kg extract showed a significant reduction in both alloxan and glucose induced hyperglycaemia [17] but the hypoglycaemic activity of the leaf had hithherto not been reported. The use of the plant locally encouraged the investigation of the leaf for its antidiabetic properties.

The leaf was extracted with methanol which is a general extracting solvent.The hypoglycaemic activity of the methanolic extract BMMet on alloxan diabetic rats was dose dependent(Table 1). The BMMet and its partition fractions, ethylacetate (BMEt), n-butanol (BMBu), and water(BMAq) at 2g/kg showed increasing hypoglycaemic activities from 1 to 4 hours, similar to that of glibenclamide (Table 1) which were significant at 2 and 4 hours. The aquaeous BMAq and Butanol BMBu fractions were the most active and were the only fractions that brought the blood glucose level to below the 200 mg/dl (11.1 mmol/L) mark (Table 1) Hyperglycaemia has been defined as a blood glucose concentration of greater than 200 mg/dl [32]). BMBu was however chosen for further purification because of its TLC profile.

Table 2: Time Course of Blood Glucose changes in Alloxan Diabetic Rats Administered the fractions of *Bauhinia monandra* leaf Butanol partitioned fractions.

Category of animal	Blood glucose level (mg/dl)			
	0HR	1HR	2HR	4HR
Normoglycaemic control	84 ± 0.6	86 ± 0.6 (-2.4%)	83 ± 0.5 (1.2%)	84 ± 0.6 (0.0%)
Diabetic negative control	341 ± 1.8	337 ± 1.6 (1.1%)	344 ± 1.7 (-1.1%)	332 ± 2.1 (3.2%)
Butanol fraction (2 g/kg)	402 ± 3.1	355 ± 3.5 (11.9%)	249 ± 1.7 (38.2%*)	131 ± 1.5 (67.6%*)
BMBuF2 (1 g/kg)	293 ± 2.6	293 ± 2.1 (0.0%)	298 ± 1.9 (-2.0%)	348 ± 3.4 (-18.7%)
BMBuF3 (1 g/kg)	307 ± 4.2	270 ± 2.8 (12.1%)	282 ± 2.1 (8.0%)	379 ± 5.0 (-23.6%)
BMBuF4 (1 g/kg)	341 ± 1.5	235 ± 2.3 (31.0%*)	284 ± 2.1 (16.7%)	305 ± 0.7 (10.3%)
BMBuF5 (1 g/kg)	360 ± 2.2	351 ± 2.6 (2.4%)	302 ± 3.1 (16.3%)	277 ± 2.6 (23.1%)
BMBuF6 (1 g/kg)	351 ± 2.7	321 ± 3.5 (8.6%)	319 ± 3.8 (9.2%)	346 ± 3.8 (1.7%)
BMBuF7 (1 g/kg)	258 ± 1.6	145 ± 1.3 (43.3%*)	118 ± 0.7 (53.9%*)	99 ± 0.2 (61.6%*)
BMBuF8 1 g/kg)	371 ± 4.0	282 ± 4.2 (23.7%)	233 ± 3.7 (37.2%)	219 ± 3.5 (40.9%*)
BMBuF9 (1 g/kg)	386 ± 4.1	274 ± 2.8 (29.1%)	226 ± 3.5 (41.5%)	169 ± 2.2 (56.2%)
BMBuF7C (750 mg/kg)	268 ± 1.7	187 ± 1.3 (30.3%)	143 ± 3.6 (46.7%)	92 ± 0.3 (65.7%*)
BMBuF7D 750mg/kg	295 ± 2.5	256 ± 2.0 (13.3%)	245 ± 1.8 (16.6%)	219 ± 1.9 (25.6%)
BMBuF7E (750 mg/kg)	293 ± 1.9	277 ± 1.6 (5.4%)	244 ± 2.5 (16.7%)	228 ± 2.6 (22.1%)
BMBuF7F (750 mg/kg)	275 ± 1.9	251 ± 2.0 (8.9%)	254 ± 2.1 (7.5%)	244 ± 2.0 (11.4%)
Diabetic positive control given Glibenclamide (5 mg/kg)	341 ± 1.8	258 ± 2.0 (24.4%*)	148 ± 0.4 (56.7%*)	109 ± 0.7 (67.8%*)

Values are statistically significant at p < 0.05; Percentages of blood glucose reduction relative to 0 hour are given in parenthesis; BMBuF2-9 and BMBuF7C - F: Bulked VLC fractions and sub fractions of butanol partitioned fraction of B. monandra leaf.*

Fractions of Butanol fraction.

The Butanol fraction fractionated into nine fractions BMBuF1-9 and all the fractions except BMBuF1and 2 were tested on alloxan diabetic rats at 1g/kg. BmBuF7 was the most active reducing hyperglycemia by 43, 54 and 61.6 % at 1, 2, and 4 hr respectively (1g/kg) (Table2), followed by BMBuF9 and 8 (56 and 41%) respectively. It is clear that the hypoglycemic constituents reside in these three fractions. Some of the fractions showed hyperglyceamic activities; BMBuF2 and F3 showed insignificant hyperglycaemic activity - 19 and - 24% respectively at the same test dose of 1 g/kg (Tables 2). Fraction BMBuF7's activity profile was similar to that of the normal dose of glibenclamide (5 mg/kg) (Tables 2). BMBuF7 was further fractionated into eight fractions of which only BMBuF7C-F were tested *in-vivo*. BMBuF7A, B, G and H were not tested due to their small weights, they were however tested *in-vitro* for insulin

release activity.BMBuF7C at a dose of 0.75g/kg showed the highest hypoglycemic activity (%) and caused the blood glucose level to reduce to a level similar to that of the normoglyceamic rats at 4hrs (Table 2). This is to be expected as when an active fraction becomes purer it is expected that its activity would increase. The remaining fractions BMBuF7D, BMBuF7E, and BMBuF7F did not show significant blood glucose lowering effect (Table 2) and the diminishing blood glucose lowering effects observed is probably due to the residual components of BMBuF7C

Table 3: Effects of *Bauhinia monandra* methanolic extract,its Ethly acetate, Butanol,Water Fractions and the Subfractions of Butanol fraction(BMBuF1-9) on glucose mediated insulin release from INS-1 cell.

Extract/Fraction/ Compound	% Insulin release (0.01 mg/ml)	% Insulin release (0.1 mg/ml)
Glucose[3.0mM]	64.5	64.5
Glucose [5.6mM]	100	100
Glucose 5.6mM+BMMet	108	121
Glucose 5.6mM+BMEt	95.6	67.4
Glucose 5.6mM+BMBu	104.3	106.5
Glucose 5.6mM+BMAq	78.2	87.7
Glucose 5.6mM+BMBuF1	121.3	236.2
Glucose 5.6mM+BMBuF2	100	95.6
Glucose 5.6mM+BMBuF3	96.7	176
Glucose 5.6mM+BMBuF4	108.7	89.1
Glucose 5.6mM+BMBuF5	97.8	94.5
Glucose 5.6mM+BMBuF6	73.9	89.1
Glucose 5.6mM+BMBuF7	83.7	154.3
Glucose 5.6mM+BMBuF8	93.5	50
Glucose 5.6mM+BMBuF9	60.8	89.1
Glucose 5.6mM+Glibenclamide (1 µg/ml)	209	209

Key-BMBuF1 - 9: Vacuum liquid chromatographic (VLC) fractions of the active butanol (BMBu) partitioned fraction of the methanolic extract of B. monandra leaf.

Table 4: Effects of Subfraction of Butanol fraction, BMBuF7 and its subfractions BMBuF7A-H on glucose mediated insulin release from INS-1 cell

/Extract/Fraction/ Compound	% Insulin release (0.01 mg/ml)	% Insulin release (0.1 mg/ml)
Glucose[3.0mM]	74.5	74.5
Glucose [5.6mM]	100	100
Glucose 5.6mM+BMBuF7	83.7	154.3
Glucose 5.6mM+BMBuF7A	129.8	119.1
Glucose 5.6mM+BMBuF7B	51.1	53.2
Glucose 5.6mM+BMBuF7C	168.1	148.9
Glucose 5.6mM+BMBuF7D	97.9	100
Glucose 5.6mM+BMBuF7E	87.2	125.5
Glucose 5.6mM+BMBuF7F	140.4	100
Glucose 5.6mM+BMBuF7G	69.1	70.2
Glucose 5.6mM+BMBuF7H	142.6	153.2
Glucose 5.6mM+Glibenclamide (1 µg/ml)	175.5	175.5

Key: BMBuF7: Vacuum liquid chromatographic (VLC) fraction of the active butanol (BMBu) partitioned fraction of the methanolic extract of B. monandra leaf; BMBuF7A-H: VLC sub fractions of the active BMBuF7 fraction.

There are various diabetic *in-vivo* models which can be used to test plants for antidiabetic activity. In this study only one model has been used based on the fact that Alloxan is known to cause diabetes by partially destroying ß-cells of the pancreas and therefore simulating type II diabetes. *In-vitro*, INS-1 cells were used to investigate the insulin stimulating activity, which is a known mechanism of action of some antidiabetic drugs. [31] .

The BMEt showed inhibition of insulin release from INS-1 cells (Table 3). The BMBu showed similar activity at both low (0.01 mg/ml) and high (0.1 mg/ml) doses while BMAq showed an inhibition of insulin release at 0.01mg/ml, which at the higher dose of 0.1mg/ml was reduced. This could mean that the water extract contains some compounds which have insulin inhibitory activity, but it contains other compounds which have a different mode of activity other than insulin release accounting for its activity *in-vivo*. It could also be that the components are metabolized *in-vivo* into other compounds which then become active (Table 3). Although BMMet and BMBu showed some ability to release insulin from the INS-1 cells, their activity was not comparable to that of glibenclamide. Glibenclamide was much more significantly active in this test. This shows that the compounds in the extract and its fractions may not act primarily by insulin release. In a very recent study kaempferol -3-O-alpha-rhamnoside obtained from *Bauhinia megalandra* leaves showed inhibition of glucose intestinal absorption [25], this suggests that there is a possibility that other constituents in the Bauhinia plant which act in the same manner.

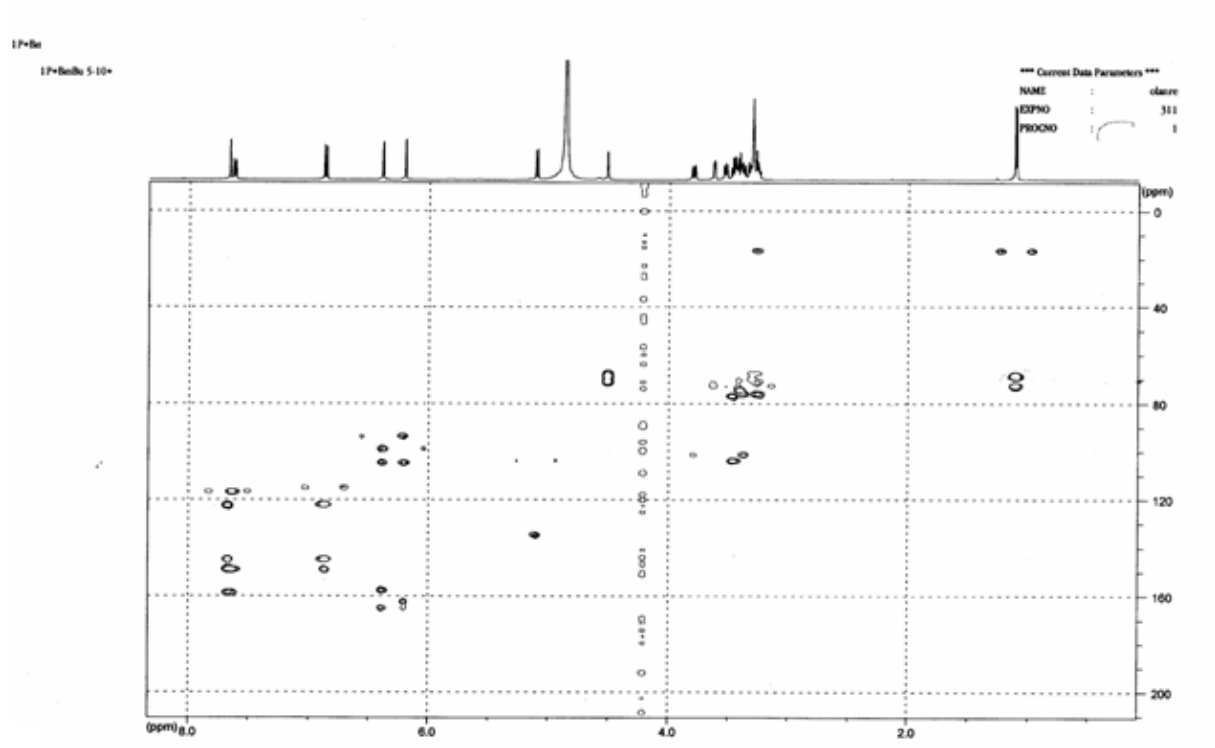
The Fractions of the BMBu which was one of the most active in-vivo were also tested for insulin release (Table 3). The most active fraction was BMBuF1 which was not assessed *in-vivo* due to its small weight. It is therefore not clear if BMBuF1 has corresponding *in-vivo* activity, this is most certainly a case for further study. The next most active was BMBuF7 showing activity of release of 54% above the control. This is consistent with its in-vivo activity but does not confirm that it mechanism of action is solely by insulin release. Fractions obtained from BMBuF7, BMBuF7A-H showed BMBuF7C and H as the most active fractions 48% and 53.3% respectively. Obviously there are different compounds showing these activities observed as the fractions in between show little activity and these fractions were obtained using different solvent mixtures. BMBuF7H being more polar than BMBUF7C.

Table 5: Effect of Quercetin-3-rutinoside from the butanol fraction of *Bauhinia monandra* leaf on glucose–mediated insulin release from INS–1 pancreatic cells

Extract/Fraction/ Compound	% Insulin release (0.01 mg/ml)	% Insulin release (0.1 mg/ml)
Glucose[3.0mM]	62.91	62.91
Glucose [5.6mM]	100	100
Glucose 5.6mM+querctin-3-rutinoside	95.4	88.5
Glucose 5.6mM+Glibenclamide (1 µg/ml)	163.2	163.2

Quercetin -3-rutinoside

The major component which was identified as Quercetin -3-rutinoside (Appendices) based on comparison of its NMR, UV, IR and MS spectra data with references [16] was found to inhibit insulin release (Table 5).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40
80
120
160
200
(ppm)
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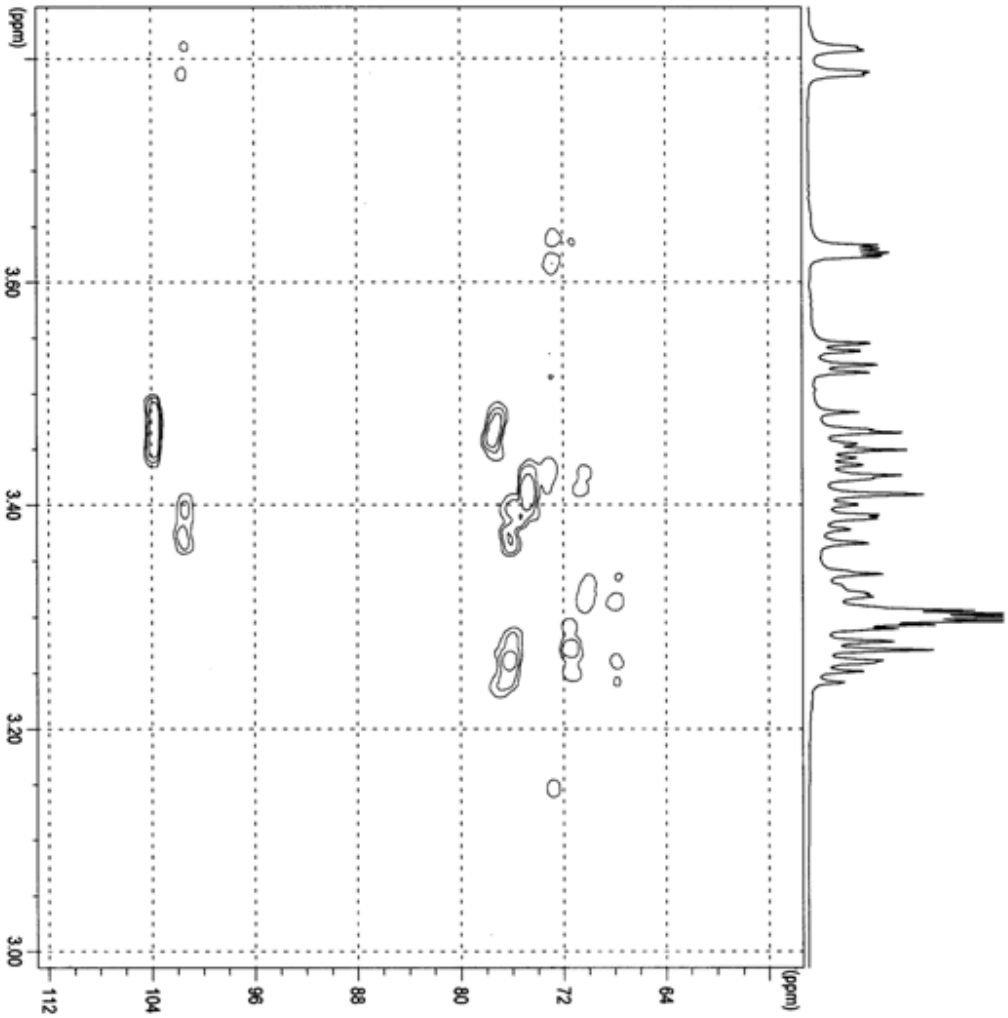
6.0
4.0
2.0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3.40
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3.00
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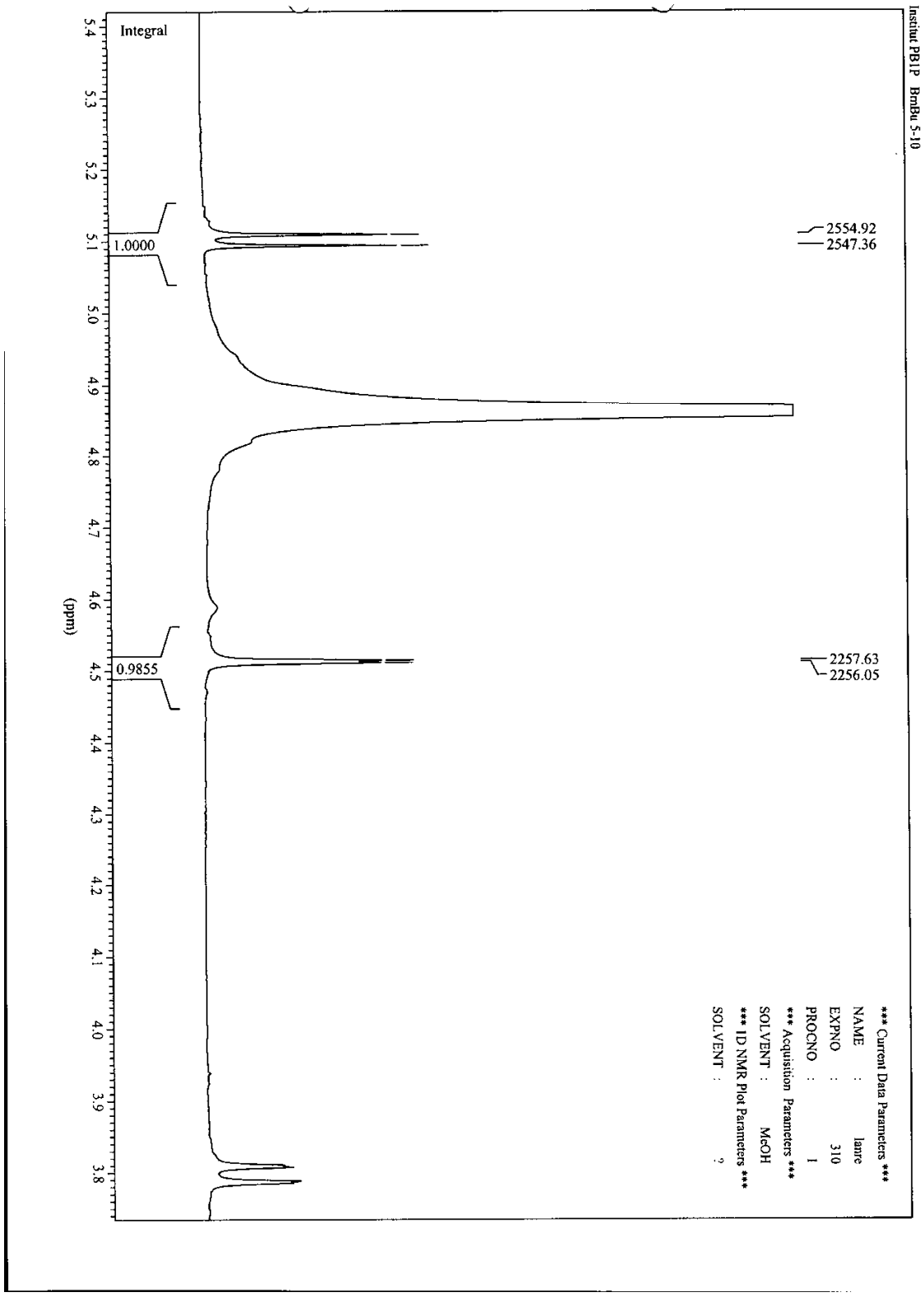
96
88
80
72
64
(ppm)


Institut PB1P BmBu 5-10
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*** ID NMR Plot Parameters ***
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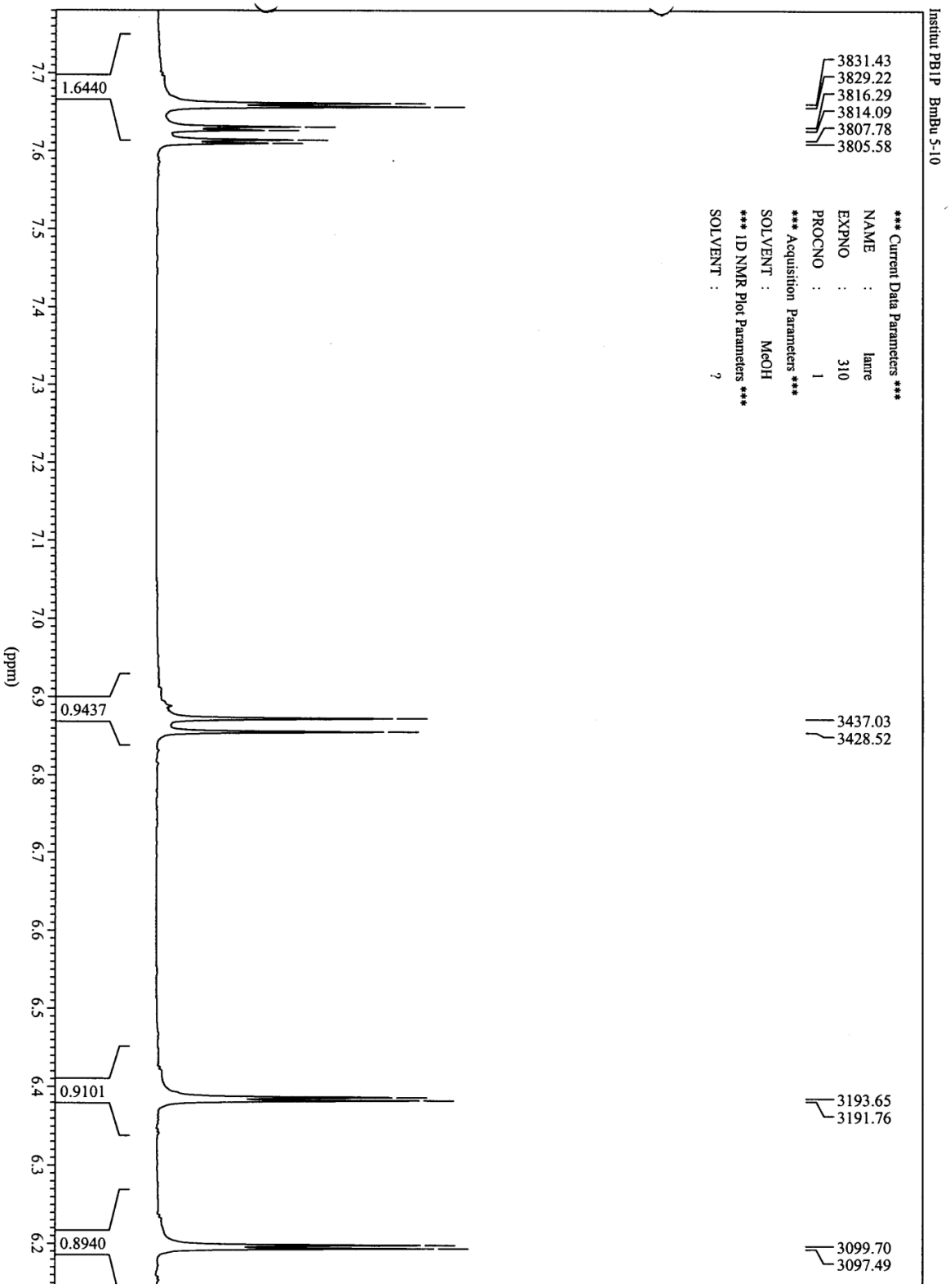
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3428.52
3193.65
3191.76
3099.70
3097.49
1.6440
0.9437
0.9101
0.8940
7.7
7.6
7.5
7.4
7.3
7.2
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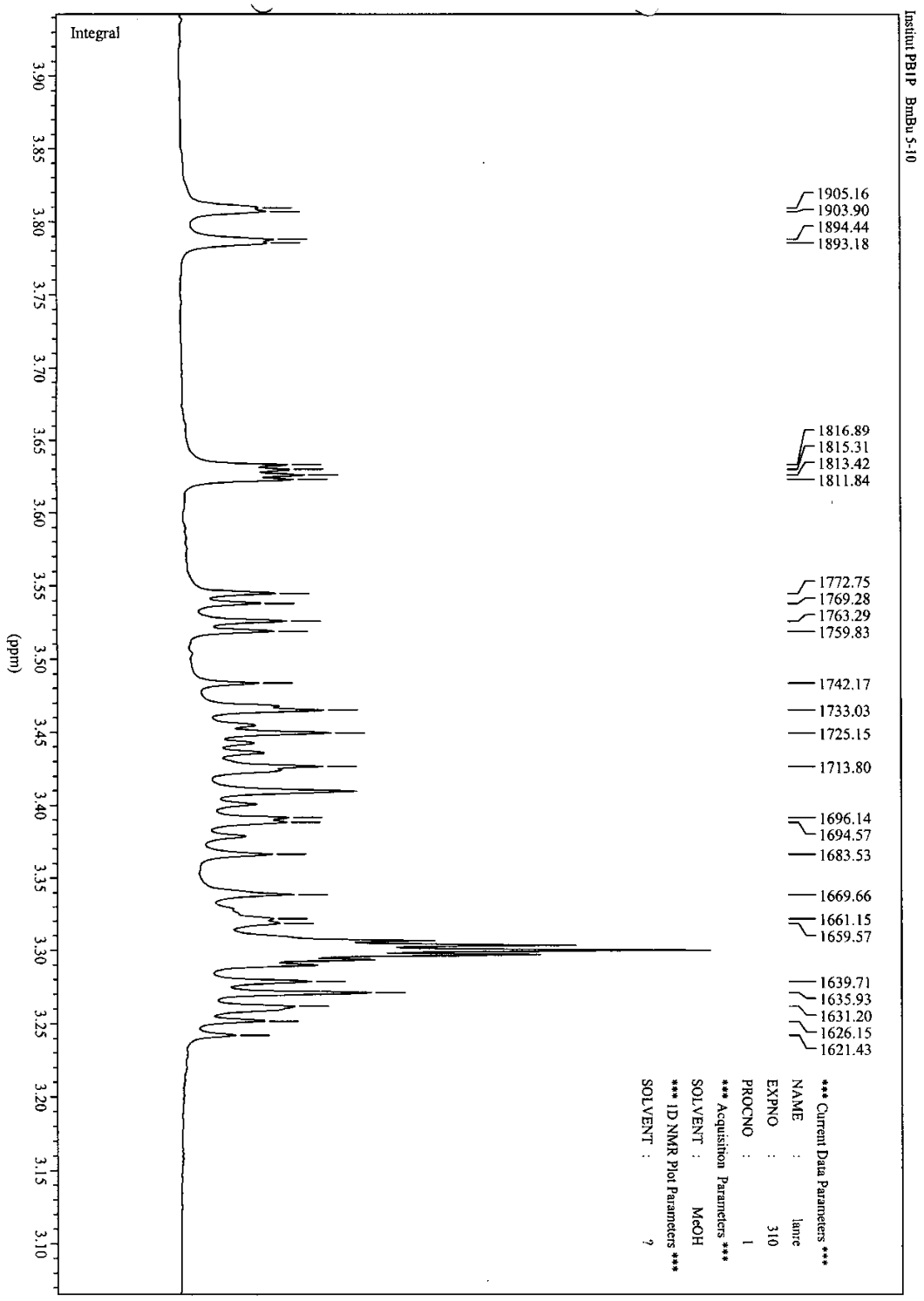
6.7
6.6
6.5
6.4
6.3
6.2
(ppm)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1769.28
1763.29
1759.83
1742.17
1733.03
1725.15
1713.80
1696.14
1694.57
1683.53
1669.66
1661.15
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1639.71
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3.70
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3.60
3.55
3.50
3.45
3.40
3.35
3.30
3.25
3.20
3.15
3.10

CONCLUSION

The similarity of activity shown by BMBuF7C (0.75g/kg), BMBuF7 (1g/kg), BMBu and BMMet(2g/kg) indicates that the components of fraction BMBuF7 are some of the principal hypoglycaemic constituents of the BMBu, BMMet and consequently of the leaf. The constituents of BMBuF1 which showed the greatest activity *in-vitro* are certainly important in the overall hypoglyceamic activity of the leaf methanolic extract.

The *in vivo* result corroborated the *in vitro* experiments and confirmed that some of the fractions and the extract had stimulating effect on the INS-1 cells leading to the release of insulin and justifies the ethnomedical use of the plant as an antidiabetic remedy.

There is no doubt that the leaves of *Bauhina monandra* has some level of hypoglyceamic activity corroborating its ethnomedicinal use. The relative safety of use of *B.monandra* leaves has also been established by toxicity studies [33]. This study has given a good basis for further investigation of the constituents of the water and butanol fractions of the methanolic extract of *B. monandra* leaves. It is pertinent that the active constituents be followed up and obtained as there is a possibility that new compounds with different modes of action may be obtained.

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