NOTE

INSULINOTROPIC PROPERTY OF SOME TROPICAL PLANT EXTRACTS ON INSULIN-SECRETING CELL LINES

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The prevalence of diabetes mellitus in Malaysia was 0.65% in 1960, 6.3% in 1986 and 8.2% in 1992 (Mafauzy 2005). In 1996, its occurrence was at an alarming stage of 10.5% of the nation's population. Diabetes not only kills, but it is also a major cause of heart attacks, strokes, blindness, gangrene and kidney failures. Presently, there is no cure for the disease but it can be controlled. Alternatively, plants have been used to treat and control this disease since ancient times. Dietary control and plant material therapies were major types of treatments for diabetes before the discovery of insulin hormone in 1922 and the development of synthetic oral hypoglycemic agents (Bailey & Day 1989). Aqueous extracts of agrimony (*Agrimony eupatoria*), alfalfa (*Medicago sativa*), coriander (*Coriandum sativum*), the edible mushroom (*Agaricus campestris*) and mistletoe (*Viscum album*) have been shown to enhance insulin secretion and imitate insulin effects on *in vitro* glucose metabolism (Gray & Flatt 1996, 1997a, b, 1998, 1999)

Tropical plants in this country have uncharted potential to be utilized for the development of novel drugs to combat diseases. Tropical plants whose extracts demonstrated insulin-releasing potential on insulin-secreting cell lines included *Tinospora crispa* (Hamdan & Ashcroft 1998) and *Gynura procumbens* (Muhajir *et al.* 2002b). We evaluated 32 methanol extracts from 15 tropical plant species for their ability to induce insulin secretion from insulin-secreting (BRIN-BD11) cell-lines. The results of this study is reported in this paper.

Powdered and dried plant parts (leaves, stem, bark and flower) were extracted using methanol for 12 hours. The methanol extracts were then concentrated in vacuum and stored at -20 °C prior to testing. BRIN BD-11 cell lines were produced by electro-fusion of the immortal RINm5F cell (Muhajir *et al.* 2002b). The cells were stored in liquid nitrogen and thawed at room temperature before use. The cells were then cultured in RPMI-1640 medium (commercial dry powder media was added to cell culture grade water and mixed with $NaHCO_3$, pH adjusted to 7.0–7.4 using NaOH or HCl) with 10% (v/v) fetal calf serum and maintained at 37 °C in CO_2 incubator.

Cell growth and confluence were observed under inverted microscope. Cells with exponential growth were seeded in culture plates at a concentration of 2.5×10^5 cells per well. Prior to seeding RPMI-1640 media supplemented with fetal serum and antibiotics was dispensed into the wells to allow attachment before test. The cells were then washed with phosphate-buffered saline (PBS) before being pre-incubated with 1 ml Krebs-Ringer bicarbonate (KRB) buffer for 40 min at 37 °C. Finally the wells were incubated for 30 min with 1ml KRB buffer and plant extracts at 37 °C. After incubation aliquots were removed from each well and stored at -20 °C for insulin secretion assay.

Insulin level was measured using commercial insulin detection kit (Mercodia AB, Sweden). Standard rat insulin was put into wells to generate a standard curve for insulin concentration determination. A total of 25 µl (0.02 mg/ml) sample was each dispensed into designated wells and incubated for two hours at room temperature in a plate shaker. Following this the micro well plate was washed and 200 µl peroxide substrate (3,3',5,5'-tetramethylbenzidine) were added and incubated for another 15 min. Lastly, 50 µl stop solution (0.5 M H_2SO_4) were added to terminate the chemical reaction. Absorbance of aliquots was measured at 450 nm and insulin concentration (µg/l) was determined from the standard insulin concentration curve. Glucose (1.1 mM) and glibenclamide (2 mM) were used as positive controls while water served as negative control.

Of the total of 32 plant extracts screened for insulin-releasing property only five showed insulinotropic (insulin releasing) activity on cell lines (Table 1). Five methanol extracts from the leaves of *Myristica fragrans*, *Piper muricatum*, *Piper* sp. and *Tabebuia chrysantha* and the fruit of *Quercus infectoria* showed promising insulin-releasing property with concentrations of 1.73100, 1.71075**,** 1.70775**,** 1.70375 and 1.72875 µg/l respectively**.**

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Table 1 Insulin concentration secreted by BRIN-BD11 cell lines after application of various plant extract test samples

Ref. No.	Plant extracts/control	Family	Plant part	Insulin concentration (µg/l)
-	Water (negative control)	-	-	0.00000 ± 0.00000
-	Glucose (positive control)	-	-	1.66925 ± 0.03392
-	Glibenclamide (positive control)	-	-	2.66900 ± 0.00940
FRI 48965	*Jacaranda obtusifolia*	Bignoniaceae	Leaf	1.67600 ± 0.01555
FRI 48965	*Jacaranda obtusifolia*	Bignoniaceae	Stem	1.67550 ± 0.01652
FRI 48977	*Myristica fragrans*	Myristicaceae	Leaf	1.73100 ± 0.01169*
FRI 48977	*Myristica fragrans*	Myristicaceae	Stem	1.68400 ± 0.01116
FRI 48967	*Peronema canescens*	Lamiaceae	Leaf	1.68225 ± 0.02209
FRI 48967	*Peronema canescens*	Lamiaceae	Stem	1.66975 ± 0.01286
DTpc/05	*Pinus caribaea*	Pinaceae	Leaf	1.67225 ± 0.01408
DTpc/05	*Pinus caribaea*	Pinaceae	Stem	1.68475 ± 0.02742
DTp1/05	*Piper* sp.	Piperaceae	Leaf	1.67050 ± 0.01948
DTp1/05	*Piper* sp.	Piperaceae	Stem	1.66625 ± 0.01564
DTp3/05	*Piper muricatum*	Piperaceae	Leaf	1.71075 ± 0.00873*
DTp3/05	*Piper muricatum*	Piperaceae	Stem	1.67500 ± 0.01505
DTp4/05	*Piper mucronatum*	Piperaceae	Leaf	1.67650 ± 0.01021
DTp4/05	*Piper mucronatum*	Piperaceae	Stem	1.68450 ± 0.04369
DTp5/05	*Piper ribesioides*	Piperaceae	Leaf	1.69450 ± 0.03688
DTp5/05	*Piper ribesioides*	Piperaceae	Stem	1.67950 ± 0.03317
DTp8/05	*Piper* sp.	Piperaceae	Leaf	1.70775 ± 0.02020*
DTp8/05	*Piper* sp.	Piperaceae	Stem	1.69825 ± 0.04252
DTQ/05	*Quercus infectoria*	Fagaceae	Fruit	1.72875 ± 0.04231*
FRI 48974	*Stenolobium smithii*	Bignoniaceae	Leaf	1.67775 ± 0.01241
FRI 48974	*Stenolobium smithii*	Bignoniaceae	Stem	1.67025 ± 0.01250
FRI 48974	*Stenolobium smithii*	Bignoniaceae	Flower	1.66250 ± 0.00759
FRI 48974	*Stenolobium smithii*	Bignoniaceae	Fruit	1.65475 ± 0.01732
FRI 48974	*Stenolobium smithii*	Bignoniaceae	Twig	1.70200 ± 0.03309
FRI 48973	*Stereospermum fibricatum*	Bignoniaceae	Leaf	1.67300 ± 0.01856
FRI 48973	*Stereospermum fibricatum*	Bignoniaceae	Stem	1.68950 ± 0.03531
FRI 48972	*Tabebuia chrysantha*	Bignoniaceae	Leaf	1.70375 ± 0.02263*
FRI 48972	*Tabebuia chrysantha*	Bignoniaceae	Stem	1.67825 ± 0.00850
FRI 48970	*Tabebuia pallida*	Bignoniaceae	Leaf	1.68225 ± 0.01129
FRI 48970	*Tabebuia pallida*	Bignoniaceae	Stem	1.67400 ± 0.01585
FRI 48964	*Tabebuia rosea*	Bignoniaceae	Leaf	1.68950 ± 0.03531
FRI 48964	*Tabebuia rosea*	Bignoniaceae	Stem	1.68075 ± 0.01521

Note: Data were presented as mean ± standard deviation (n = 4). Data analyzed by Student's *t*-test and ANOVA ($p < 0.05$).
* Significant differences compared with glucose control

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