Antidiabetic screening and scoring of eleven plants traditionally used in South Africa

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Abstract

Ethnopharmacological relevance: The multitude of metabolic steps affected by Type II diabetes offer many drug targets but they complicate *in vitro* screening to validate traditional uses or find new drug leads from plants. **Aim:** To investigate the traditional antidiabetic uses of indigenous or naturalised South African plants using an optimised screening and scoring method. **Materials and Methods:** Eleven plant species were screened against Chang liver, 3T3-L1 adipose and C2C12 muscle cells measuring glucose utilisation in all three cell lines and toxicity in hepatocytes and myocytes. A scoring system was devised to aid interpretation of results. **Results:** *Catharanthus roseus* results correlated with previously reported *in vivo* results, with best stimulation of glucose utilisation in hepatocytes (this sounds confusing as earlier you indicate that hepatocytes were used for toxicity testing). *Momordica foetida* and *M. balsamina* extracts were active in myocytes but only the latter stimulated glucose utilisation in hepatocytes (this sounds confusing as earlier you indicate that hepatocytes were used for toxicity testing).. *Brachylaena discolor* gave the best overall results, with all plant parts giving high activity scores and negligible toxicity. *In vitro* toxicity results for *Catharanthus roseus, Vinca major, Momordica balsamina* and some *Sclerocarya birrea* extracts raise concern for chronic use. **Conclusion:** This screening system increases the likelihood of identifying drug candidates using *in vitro* antidiabetic screening of crude plant extracts, whilst the scoring system aids data interpretation.

(197 words)

Key words: 3T3-L1, C2C12, Chang liver, Glucose utilisation, Antidiabetic, Medicinal plants, South Africa

1. Introduction

Type II diabetes affects many metabolic pathways in different tissues, many of which are potential targets for drug treatment. Unfortunately, this complicates the identification of new treatments, as most *in vitro* screening models consider a single cell type, metabolic pathway or enzyme, thus greatly reducing the possibility of identifying an antidiabetic extract or compound. Another disadvantage of these methods is that only "acute" or immediate effects are measured, whilst effects that may only be apparent after chronic exposure to the antidiabetic compound are overlooked. In this regard, animal models are more useful but ethical and practical considerations make it impossible to screen large numbers of samples.

Type II diabetes is characterised by hepatic and peripheral (muscle, adipose tissue) insulin resistance. The pancreas compensates by secreting more insulin, but eventually the beta cells will fail to sustain this (Cerasi, 2000), at which stage the patient requires insulin treatment. During the stage when insulin is still produced, various other classes of drugs, in combination with lifestyle alterations, can be used to manage the disease (Matthaei et al., 2000). These drugs act through a number of different ways or targets to reduce blood glucose levels. When screening for an antidiabetic agent, it is important to include as many of these targets as possible to ensure that possible 'hits' are not excluded.

South Africa has remarkable biodiversity and rich cultural traditions of plant use. Scientific understanding of medicinal plants is, however, largely unexplored and

pharmacological investigation of the South African flora only gained momentum recently (Fourie et al. 1992; Van Wyk, 2002). In light of this and the pressing need for new antidiabetic agents, the Innovation Fund of the Department of Science and Technology of South Africa awarded funds to a consortium comprising of several national institutions to evaluate local medicinal plants used for various therapeutic properties, including diabetes.

In this study 11 plant species, used traditionally in South Africa to treat diabetes, were selected based on literature reports. Aqueous and organic extracts of the plants were screened against Chang liver, C2C12 muscle and 3T3-L1 adipose cells using a glucose utilisation assay. The results were interpreted using a scoring system devised to consider the potential antidiabetic activity as well as toxicity to assist in selection of the most active and least toxic extracts. Where possible, the results were compared to previously published work performed on animal models to validate the screening and scoring system.

2. Materials and Methods

2.1 Plant selection:

From a flora of approximately 20 000 taxa it was necessary, given logistical constraints, to identify those most likely to exhibit antidiabetic activity. Following a search in the ethnomedicinal literature for the terms “diabetes” and “blood sugar”, a total of 28 taxa were identified as being used whole or in part, or in polycomponent preparations, for the

treatment of diabetes. In order to select the most promising taxa for assaying, these 28 taxa were ranked following the application of weighted criteria for general ethnomedicinal use (including such elements as popularity in ethnomedicinal trade) and toxicity. Plants reportedly toxic were accorded extra weight as toxicity was taken as an indicator of bioactivity.

A similar semi-quantitative selection method has previously been applied to identify and rank antiplasmodial plant candidates from South Africa (Clarkson et al., 2004). From the ranked list of a total of 28 taxa in the current study, more than 70% attained total scores of ≥13 out of a possible maximum 24 points, the highest score being 16.5. Eleven species were collected throughout South Africa and subsequently investigated for antidiabetic activity. The identity of voucher specimens was confirmed by the National Herbarium of the South African National Biodiversity Institute (PRE) where they were deposited.

2.2 Preparation of plant extracts:

Plant parts from each sample were separated and dried in an oven at 30–60°C. Drying time and temperature varied depending on the nature of the plant part. Dried plant material was ground to a coarse powder using a hammer mill and stored at ambient temperature prior to extraction. For each extraction procedure, 100–500 g of powdered plant material was sequentially extracted with cold dichloromethane (DCM): methanol (MeOH) (1:1) and purified water. Organic extracts were concentrated by rotary vacuum evaporation below 45 °C and further dried *in vacuo* at ambient temperature for 24 h. The

aqueous extracts were concentrated by freeze-drying. All dried extracts were stored at −20°C.

2.3 Antidiabetic and toxicity screening:

Dried plant extracts were reconstituted in undiluted DMSO, vortexed and left for at least 15 minutes before further dilution with the respective growth medium for chronic exposure or incubation medium for acute exposure. The final DMSO concentration was less than 0.25%. Undiluted DMSO stock solutions were stored at 4°C for a maximum of 6 weeks.

Murine C2C12 myoblasts and 3T3-L1 preadipocytes as well as human Chang liver cells were used for screening. Cells were used within ten passage numbers to limit batch-to-batch variation. Growth medium for the Chang liver and C2C12 cell lines included RPMI 1640 (Highveld Biological, South Africa) with 10% foetal bovine serum (Highveld Biological, South Africa), whereas 3T3-L1 cells were cultured in DMEM (1.5 g/L $NaHCO_3$) (Highveld Biological, South Africa) with 10% foetal bovine serum. C2C12 myoblasts were seeded at a density of 5 000 cells per well into 96-well culture plates (Nunc, Denmark) and cultured for 3-4 days. Chang liver and 3T3-L1 preadipocytes were seeded at 6 000 and 3 000 cells per well, respectively, and cultured for 5 days in growth medium to allow formation of confluent monolayers of the three cell lines. C2C12 and 3T3-L1 cells also differentiated during this time, as was evident from the formation of myotubules in C2C12 cells and the accumulation of fat droplets in 3T3-L1 cells. Longer culture times resulted in C2C12 cell death and 3T3-L1 cells becoming difficult to

manipulate because of their high buoyancy. After seeding, cells were not fed until the day of the glucose utilisation experiment to allow for depletion of glucose from the medium.

Positive controls used were 1 µM insulin for C2C12 and 3T3-L1 cells and 1 µM metformin for Chang liver cells. These concentrations gave maximum stimulation of glucose utilisation in the respective cell lines (results not shown). The acute effect of extracts was observed in C2C12 cells by exposing the cells to the extracts on the day of experimentation. Chronic effects were determined in Chang liver and 3T3-L1 preadipocytes by exposing the cells to extract or metformin for 48 hours prior to, and again during, the glucose utilisation experiment. This was done by adding 10 µl of extract or metformin to the medium in the respective wells to yield final concentrations of 12.5 µg/ml of extract, or 1 µM metformin, without changing the culture medium. Cells were not pre-exposed to insulin to prevent insulin resistance. Following the 48 hour exposure, cell viability in representative wells was assessed using the MTT assay (Mosmann, 1983). To compensate for differences in cell number due to chronic exposure of Chang liver and 3T3-L1 cells to plant extracts, the MTT results were used to normalise the glucose utilisation results.

For the glucose utilisation experiment, all procedures were completed at 37°C. Fifty (50) µl of incubation medium (8 mM glucose RPMI 1640 + 0.1% BSA) containing the specific treatment (1 µM insulin or metformin as positive controls or 50 µg/ml extract) was added to the appropriate wells. Control wells contained incubation medium only. After incubation (3 hours for Chang liver, 1.5 hours for 3T3-L1 and 1 hour for C2C12

cells), 10 µl was removed from each well and placed into a new 96-well plate to which 200 µl glucose oxidase reagent (SERA-PAK Plus, Bayer) was added. The plates were further incubated for 15 minutes at 37°C and the absorbance was measured at 492 nm using a Multiscan MS microtiter plate reader (Labsystems). To calculate glucose utilisation, the amount of glucose left in the medium after incubation was subtracted from the initial amount (8 mM).

3. Results

Data from a previous study performed in our laboratory have shown that optimal results are obtained when Chang liver and 3T3-L1 adipose cells are exposed to a low concentration of plant extract (12.5 µg/ml) for 48 hours before the glucose utilisation experiment using 50 µg/ml extract. C2C12 myocytes, on the other hand, required no pre-exposure to the extract (unpublished results). The 48 hour pre-exposure of Chang liver and 3T3-L1 cells allow the simultaneous measurement of cell viability in representative wells to determine the toxicity of the extract on the cells.

From the 11 plant species that were selected, a total of 41 extracts were prepared from various plant parts. The organs of a single plant often possess different secondary metabolite profiles owing to their specialised form and function. An aqueous and an organic (dichloromethane:methanol, 1:1) extract was prepared from each plant part, with a few exceptions where insufficient material was available. The glucose utilisation and toxicity results obtained for Chang liver cells are shown in Figure 1. When the results

were considered for all three cell lines, it was difficult to identify the most promising extracts by analysing the graphs and therefore a scoring system was devised. All results for the three cell lines were expressed as percentage of control, as in Figure 1 for Chang liver cells. The response of untreated control cells was taken as 100%. The mean responses obtained from six individual experiments with positive controls gave the following results in the respective cell lines: 1 µM metformin in Chang liver cells: 149.5 ± 16.5%; 1 µM insulin in 3T3-L1 cells: 158.7 ± 10.7% and in C2C12 cells: 156.7 ± 11.2% (mean ± SEM, N = 6 experiments, with 8 (Chang liver and 3T3-L1) or 12 (C2C12) replicate wells per experiment).

Scoring criteria were established using the responses observed in the respective positive controls in each cell line, as well as the range of responses obtained with the different extracts (Table 1). A response <120% of control was allocated a score of 0; a response between 120% and the mean response of the respective positive control scored +1 and any response greater than that of the positive control, +2. The criteria were applied to all three cell lines. The sum of the three activity scores yielded a maximum achievable activity score of +6. Negative scores were given for toxicity because treatments for Type II diabetes are taken for long periods and therefore any toxic effect is undesirable. As no guidelines could be found in the literature with respect to *in vitro* toxicity of plant extracts used for the treatment of chronic conditions such as diabetes, statistical significance was used as a guideline. Initially, values that were lower than control values at the level of $p<0.05$ were scored -1 and those with $p<0.001$ were scored -2. For the majority of extracts (more than 90%), this correlated to <90% ($p<0.05$) and <80%

($p<0.001$) of the control; subsequent scoring was based on these percentages (Table 1). As toxicity was measured only in two of the three cell lines, the lowest achievable toxicity score was -4. Toxicity scores were subtracted from activity scores to give a final overall score.

The glucose utilisation results for 3T3-L1 and C2C12 and the toxicity results for 3T3-L1 are not shown; however, the data were used for applying the scoring criteria summarised in Table 2. The total activity scores of the 41 extracts ranged between 0 and +6, of which only the organic leaf extract of *Vinca major* scored the maximum of +6. After subtracting the toxicity scores, however, the highest overall score was +4. Four extracts were unable to stimulate glucose utilisation in at least one of the cell lines: the organic root extract of *Sclerocarya birrea*, the organic leaf extract of *Cissampelos capensis* and the organic and aqueous root extracts of *Catha edulis.* It was noted that some extracts caused a high increase in glucose utilisation in only one cell type, while others had an effect on more than one cell type. For example, the *Momordica foetida* organic extract scored +2 in C2C12 muscle cells but scored 0 in the other cell types. In contrast, the *V. major* organic leaf extract strongly stimulated glucose utilisation in all three cell types. This observation was not unexpected, considering the different mechanisms of glucose uptake and metabolism in different tissue types. Indeed, it illustrates the necessity of a screening system that represents all the major target tissues for antidiabetic screening. In many instances, very good glucose utilisation results were recorded but due to toxicity, the final overall scores were relatively low (e.g. most of the *M. balsamina* and *V. major*

extracts). *Brachylaena discolor* gave exceptional results, with all six extracts showing good glucose utilisation activity and no or very low toxicity scores.

4. Discussion

Some of the plant species screened in this study have previously been tested against diabetes in animal models. These results were used to evaluate our screening and scoring system. Extensive literature is available on the bioactivity of *C. roseus*. The sap of fresh leaves was shown to reduce blood glucose in alloxan-treated rabbits (Nammi *et al*., 2003). Singh *et al*. (2001) have shown antidiabetic activity of a dichloromethane:methanol (1:1) extract of flowering twigs in streptozotocin (STZ)-induced diabetic rats. A significant increase in glucokinase activity was reported in the livers of rats treated with the extracts and the authors stated that an increase in glucose utilisation could be the mechanism of antidiabetic activity of this extract. This correlates well with our findings, viz. where organic leaf and twig extracts scored +2 in Chang liver cells. Although toxicity was not observed in the diabetic rats treated with *C. roseus* extract (Singh *et al*., 2001), the duration of treatment was short and the authors suggested that chronic toxicity should be investigated for human use. Our results indicate that there was toxicity in Chang liver and 3T3-L1 cells after a 48 hour exposure to relatively low extract concentrations of 12.5 µg/ml. Over 75 alkaloids are produced by this species (Garub-Fakim, 2006) and many of them have been shown to interfere with microtubule function (Kruczynski and Hill, 2001). It is possible that these alkaloids were responsible for the toxic effects observed in this study.

Rau *et al*. (2006) reported that an ethanolic extract of *C. roseus* activated the nuclear peroxisome proliferator activated receptors PPARγ, PPARα and PPARδ in cultured human cells. These nuclear receptors function as transcription factors upon activation and regulate the expression of genes that ultimately control lipid and glucose homeostasis and adipocyte differentiation. In this study, the organic extracts of the twigs and leaves strongly stimulated glucose utilisation in 3T3-L1 adipocytes.

A dichloromethane:methanol (1:1) extract of *S. birrea* bark decreased blood glucose and increased plasma insulin levels in STZ rats (Dimo *et al*., 2007). A significant improvement was seen in glucose tolerance during an oral glucose tolerance test in diabetic rats treated with the extract. The authors speculated that this improvement could be associated with stimulation of insulin secretion. Our results showed that the organic bark extract caused a marked increase in glucose utilisation in Chang liver cells and in C2C12 muscle cells (score +2 for both), suggesting a different mechanism to that proposed by Dimo *et al*. (2007). The toxicity of this extract resulted in the overall score of +1 despite its positive effects on glucose utilisation; this may raise concerns about the medicinal use of the plant. Methanolic and aqueous bark extracts of *S. birrea* had high LD50 values in mice (median LD50 value 1215 ± 38 mg/kg), suggesting no acute toxicity (Ojewole, 2003). However, the author stated that some chemical constituents previously identified in these extracts could be potentially toxic to mammals and our results show *in vitro* toxicity. The differences in activity of the different parts of the plant are noteworthy, with the organic stem extract being the most active and at the same time it appeared to be non toxic, yielding an overall score of +4.

The organic leaf extract of *Catha edulis* (khat) scored +4 overall due to moderate stimulation of glucose utilisation in fat and muscle cells and no toxicity (Table 2). Fresh leaves are chewed in many parts of Africa for their stimulant effect, and the plant has been claimed to lower blood glucose levels in diabetes. The opposite effect has been shown in a study by Saif-Ali *et al*. (2003) where an insignificant increase in blood glucose was accompanied by a significant increase in plasma C-peptide in Type II diabetic khat chewers. The effect is thought to be an indirect sympathomimetic action (Saif-Ali *et al*., 2003) which cannot be reflected in our *in vitro* screening system. The moderate effect observed on glucose utilisation in our results is probably masked *in vivo* by the opposing effects observed by Saif-Ali *et al*. (2003).

The extracts of the two species of *Momordica* tested in this study had final scores ranging between +1 and +3 and most showed some degree of toxicity (Table 2). A previous study by Marquis *et al*. (1977) has shown that foetidin, isolated from *M. foetida*, lowered blood glucose levels in normal but not in diabetic rats. *M. charantia* Descourt. has been shown to have hypoglycaemic activity in a variety of diabetic animal models (Akhtar *et al*., 1981; Cakici *et al*., 1994; Leatherdale *et al*., 1981; Tennekoon *et al*., 1994; Welihinda and Karunanayake, 1986) and possible toxic effects, including hepatotoxicity, have been reported (Basch *et al*., 2003; Tennekoon *et al*., 1994). A significant increase in glycogen levels in treated diabetic rats suggests that the liver is the target organ or at least one of the target organs or *M. charantia* (Sarkar *et al*., 1996) and *M. cymbalaria* Fensl. ex Naud. (Kameswara Rao *et al*., 1999). Our results confirmed that *M. balsamina* extracts,

particularly organic extracts, possess activity in hepatocytes. All the extracts of *M. foetida* and *M. balsamina* were active in muscle cells.

Numerous studies have reported hypoglycaemic activity of *Psidium guajava* extracts. Aqueous leaf extracts were tested in STZ-induced (Ojewole, 2005) and in alloxan-induced diabetic rats (Mukhtar *et al*., 2004), an ethanolic extract of stem bark was tested on alloxan-induced hyperglycaemic rats (Mukhtar *et al*., 2006), and a butanol-soluble fraction of the leaves was tested on $Lepr^{db}/Lepr^{db}$ mice (Oh *et al*., 2005). In this study, the organic leaf and root extracts as well as the aqueous root extract were active in fat and muscle cells but the activity was accompanied by toxicity in Chang liver and 3T3-L1 fat cells (Table 2). A recent study has shown significant inhibition of alpha-glucosidase activity in the small intestine of diabetic mice (Wang *et al*., 2007). Inclusion of an assay for *in vitro* alpha-glucosidase inhibition would enhance the screening system reported here, by identifying plant extracts that may slow down carbohydrate digestion *in vivo*.

The effects of other plant species screened, several of which showed promising bioactivity, could not be verified against literature reports of testing in animal models of diabetes. This is especially true for all extracts of *B. discolor* and the aqueous extracts of *Chironia baccifera* and *Cissampelos capensis* where the absence of *in vitro* toxicity is very encouraging. Most of the *V. major* extracts were toxic, probably due to the presence of alkaloids.

The antidiabetic screening method described here was designed to optimise a screening test for plant extracts and facilitate the interpretation of results by applying a scoring system. In this way, plant species with promising antidiabetic activity can be effectively screened and selected for further investigations, including the isolation of active compounds. Bioactivity screening, furthermore, may result in the validation of traditionally used plant species, so recognising the cultural, medical and economic importance of such taxa.

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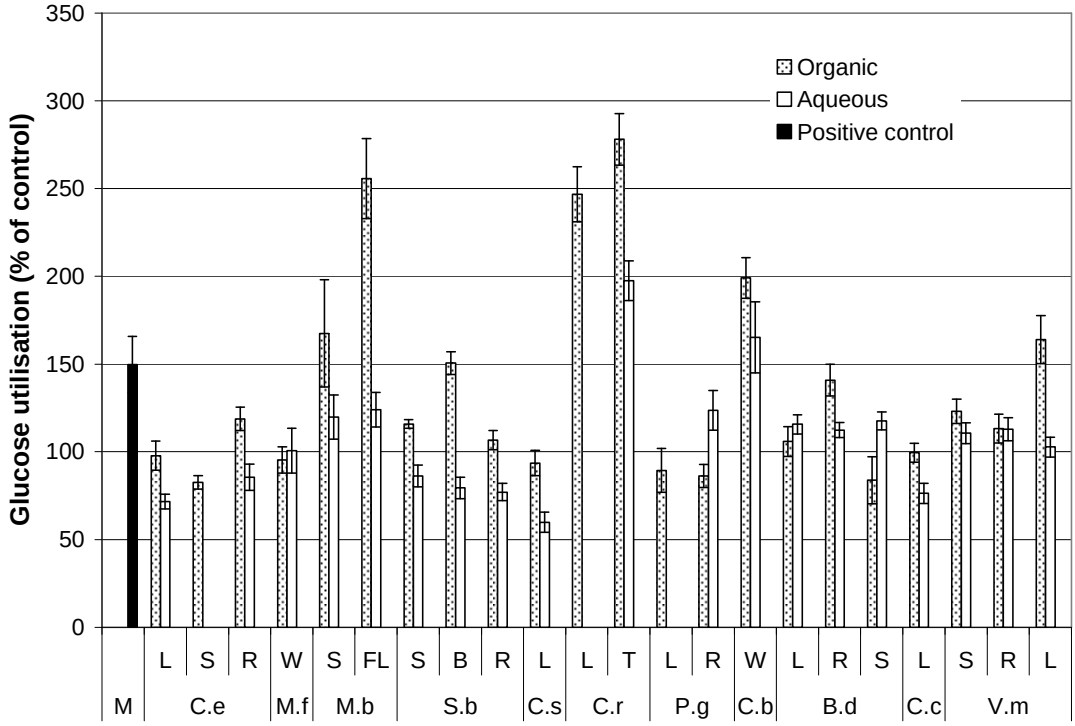
Glucose utilisation (% of control)
350
300
250
200
150
100
50
0
Organic
Aqueous
Positive control
L S R W S FL S B R L L T L R W L R S L S R L
M C.e M.f M.b S.b C.s C.r P.g C.b B.d C.c V.m

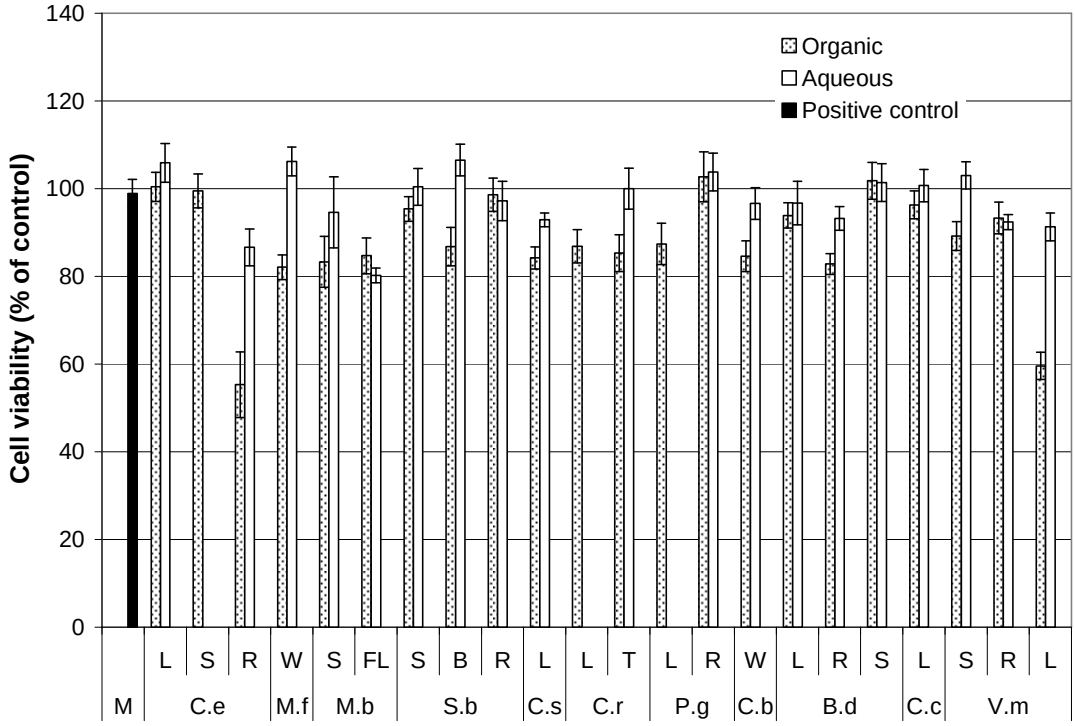
Cell viability (% of control)
140
120
100
80
60
40
20
0
Organic
Aqueous
Positive control
L S R W S FL S B R L L T L R W L R S L S R L
M C.e M.f M.b S.b C.s C.r P.g C.b B.d C.c V.m

Figure 1. Effect of plant extracts and the positive control, metformin (M) on glucose utilisation (top) and toxicity (bottom) in Chang liver cells. Cells were exposed to 12.5 µg/ml extract or 1 µM metformin for 48 hours, whereafter glucose utilisation was measured in the presence of 8 mM glucose and 50 µg/ml extract or 1 µM metformin for 3 hours. Organic extracts were prepared with dichloromethane:methanol (1:1). B = bark; F = flowers; L = leaves; R = roots; S = stems; T = twigs; W = whole plant. The first letters of genus and species names of each plant were used to abbreviate plant names; refer to Table 2 for full names. Data points represent mean ± SEM; N = 8 (glucose utilisation) or 4 (cell viability) wells in a 96-well plate.

Table 1. Scoring system used to evaluate the overall potential of each extract to increase glucose utilisation in different cell types without adverse toxic effects.

Cell line	Glucose utilisation score[a]			Toxicity score[a]		
	0	+1	+2	0	-1	-2
Chang liver	<120%	120-150%	>150%	90% +	80-89%	<80%
3T3-L1 adipocytes	<120%	120-159%	>159%	90% +	80-89%	<80%
C2C12 myocytes	<120%	120-157%	>157%	n/a	n/a	n/a

[a] Refers to % of untreated control, where the glucose utilisation and viability of untreated control cells were taken as 100%.

n/a: Not applicable, C2C12 cells were not pre-exposed to extract and toxicity was not measured on this cell line.

Table 2. Summary of the effect of plant extracts on glucose utilisation and toxicity in Chang liver, 3T3-L1 adipose and C2C12 muscle cells using a scoring system as described in Table 1.

Species	Plant part[a]	Extract type[b]	Chang liver		3T3-L1		C2C12	Activity score (max +6)	Toxicity score (min -4)	Total overall score
			Active	Toxic	Active	Toxic	Active			
Catha edulis (Vahl) Forrsk. ex	L	Org	0	0	2	0	2	**4**	**0**	**4**
Endl.		Aq	0	0	0	0	1	**1**	**0**	**1**
Celastraceae	S	Org	0	0	1	0	0	**1**	**0**	**1**
	R	Org	0	-2	0	0	0	**0**	**-2**	**-2**
		Aq	0	-1	0	-1	0	**0**	**-2**	**-2**
Momordica foetida Schumach.	W	Org	0	-1	0	0	2	**2**	**-1**	**1**
Cucurbitaceae		Aq	0	0	0	0	1	**1**	**0**	**1**
Momordica balsamina L.	S	Org	2	-1	0	-1	2	**4**	**-2**	**2**
Cucurbitaceae		Aq	1	0	0	-1	1	**2**	**-1**	**1**
	FL	Org	2	-1	1	-1	1	**4**	**-2**	**2**
		Aq	1	-1	2	0	1	**4**	**-1**	**3**
Sclerocarya birrea (A.Rich.)	S	Org	0	0	2	0	2	**4**	**0**	**4**
Hochst. subsp. *caffra* (Sond.)		Aq	0	0	0	0	2	**2**	**0**	**2**

Kokwaro	B	Org	2	-1	0	-2	2	**4**	**-3**	**1**
Anacardiaceae		Aq	0	0	0	0	2	**2**	**0**	**2**
	R	Org	0	0	0	0	0	**0**	**0**	**0**
		Aq	0	0	2	-1	0	**2**	**-1**	**1**
[c]*Cannabis sativa* L.	L	Org	0	-1	2	0	0	**2**	**-1**	**1**
Cannabaceae		Aq	0	0	2	0	0	**2**	**0**	**2**
Cissampelos capensis L.f.	L	Org	0	0	0	0	0	**0**	**0**	**0**
Menispermaceae		Aq	0	0	2	0	0	**2**	**0**	**2**
[c]*Catharanthus roseus* (L.)	L	Org	2	-1	2	-1	1	**5**	**-2**	**3**
G.Don.	T	Org	2	-1	2	-1	0	**4**	**-2**	**2**
Apocynaceae		Aq	2	0	1	-1	0	**3**	**-1**	**2**
[c]*Psidium guajava* L.	L	Org	0	-1	2	-1	1	**3**	**-2**	**1**
Myrtaceae	R	Org	0	0	2	-1	1	**3**	**-1**	**2**
		Aq	0	0	2	-2	1	**3**	**-2**	**1**
Chironia baccifera L.	W	Org	2	-1	1	-2	0	**3**	**-3**	**0**
Gentianaceae		Aq	2	0	1	0	1	**4**	**0**	**4**

Brachylaena discolor DC.	L	Org	0	0	2	0	2	**4**	**0**	**4**
Asteraceae		Aq	0	0	2	0	2	**4**	**0**	**4**
	R	Org	1	-1	2	0	2	**5**	**-1**	**4**
		Aq	0	0	2	0	2	**4**	**0**	**4**
	S	Org	0	0	2	0	2	**4**	**0**	**4**
		Aq	0	0	2	0	2	**4**	**0**	**4**
[c]*Vinca major* L.	S	Org	1	-1	2	-1	2	**5**	**-2**	**3**
Apocynaceae		Aq	0	0	2	-2	2	**4**	**-2**	**2**
	R	Org	0	0	1	-2	1	**2**	**-2**	**0**
		Aq	0	0	2	-2	2	**4**	**-2**	**2**
	L	Org	2	-2	2	0	2	**6**	**-2**	**4**
		Aq	0	0	2	0	2	**4**	**0**	**4**

[a] B = bark; F = flowers; L = leaves; R = roots; S = stems; T = twigs; W = whole plant.
[b] Aq – aqueous extract; Org - organic extract prepared with dichloromethane:methanol (1:1).
[c] Exotic to South Africa