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Antihiperglycaemic Activity of *Bauhinia megalandra*

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1. Introduction

Glycaemia is a homeostatic parameter that is very efficiently controlled thanks to the equilibrium that exists between the mechanisms involved in supply and removal of blood glucose (Fig. 1).

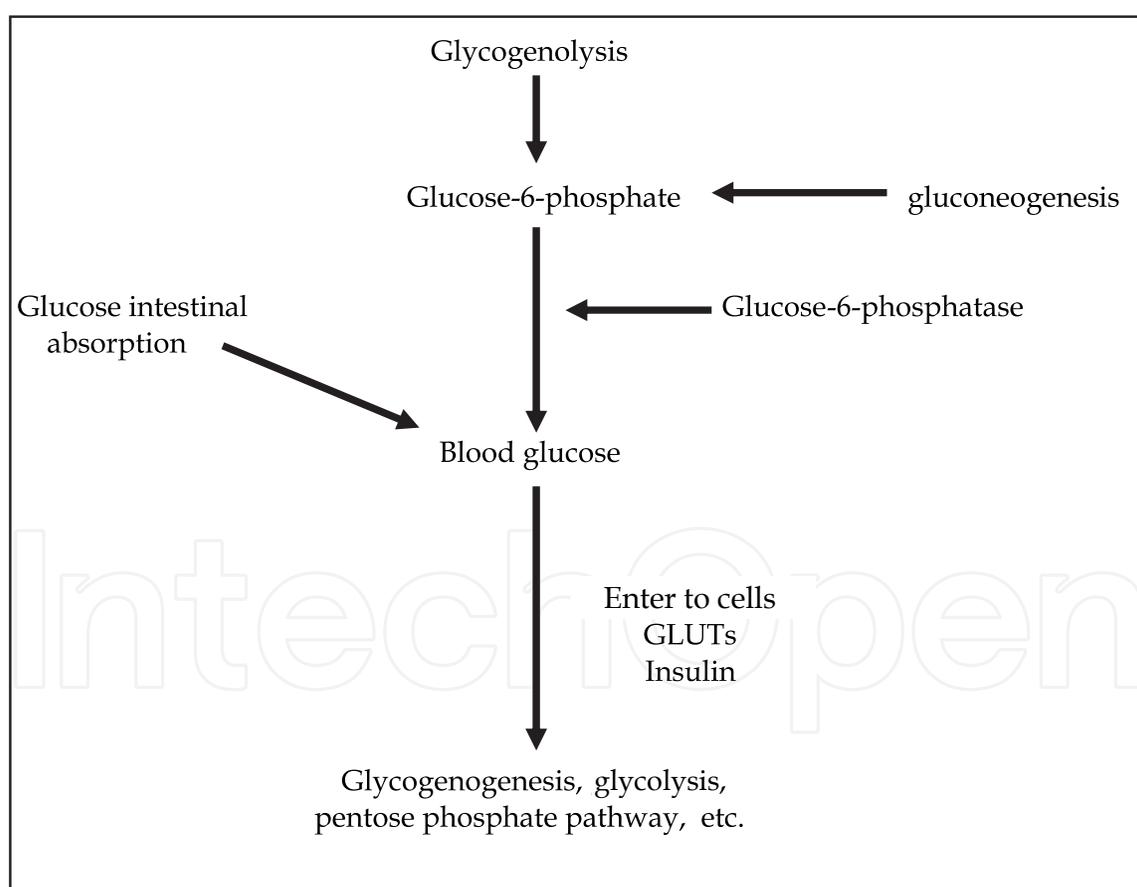


Fig. 1. Control of glycaemia. Plasma glucose concentration is closely controlled thanks to equilibrium between the mechanisms that supply glucose to the blood and remove it.

The intestinal absorption is one of the mechanisms that supplies glucose to the blood and occurs in two steps (Fig. 2): the first is mediated by the Na^+ -glucose transporter (SGLT1)

located at the apical membrane of the enterocyte and it translates 2 Na⁺ ions in favour of its concentration gradient per molecule of glucose moved in favour or against its concentration gradient into the cell, depending on the amount of the carbohydrate ingested and the time of digestion. The second step is carried out by the glucose transporter 2 (GLUT2) situated at the basolateral membrane of the enterocyte and this is able to transport glucose by a facilitated diffusion in or out of the cell (Nelson & Cox, 2005).

During a short fasting period, hepatic glycogen is degraded (glycogenolysis) in order to supply glucose to the blood. If the fasting period is prolonged a new synthesis of glucose occurs (gluconeogenesis) especially in liver and kidney. The final product of both processes is glucose-6-phosphate which is hydrolyzed to glucose and phosphate by the enzyme glucose-6-phosphatase (EC 3.1.3.9), allowing the glucose to exit the cell and enter the blood. In consequence this enzyme plays an important role in controlling the glycaemia (Ashmor and Weber 1959). The glucose-6-phosphatase is located in the endoplasmic reticulum (80-90 %) and nuclear envelope (10-20 %) mainly in the liver, kidney, enterocyte and β cells of pancreatic islets. Based on kinetic, genetic and molecular studies, Arion *et al.*, (1975) and Burchell & Waddell (1991) postulated that the glucose-6-phosphatase is constituted by (Fig. 3): A transmembrane protein, the catalytic subunit, whose active centre faces the cistern of the endoplasmic reticulum and shows a low specificity, being able to hydrolyze several phosphoric esters and pyrophosphate.

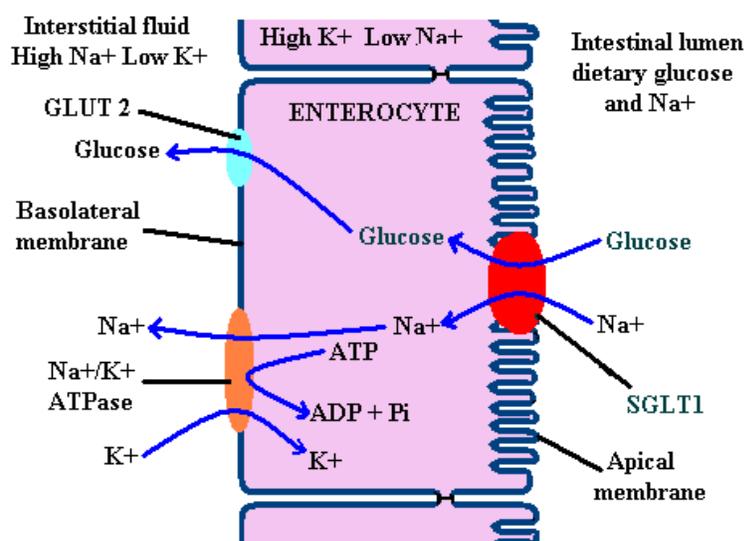
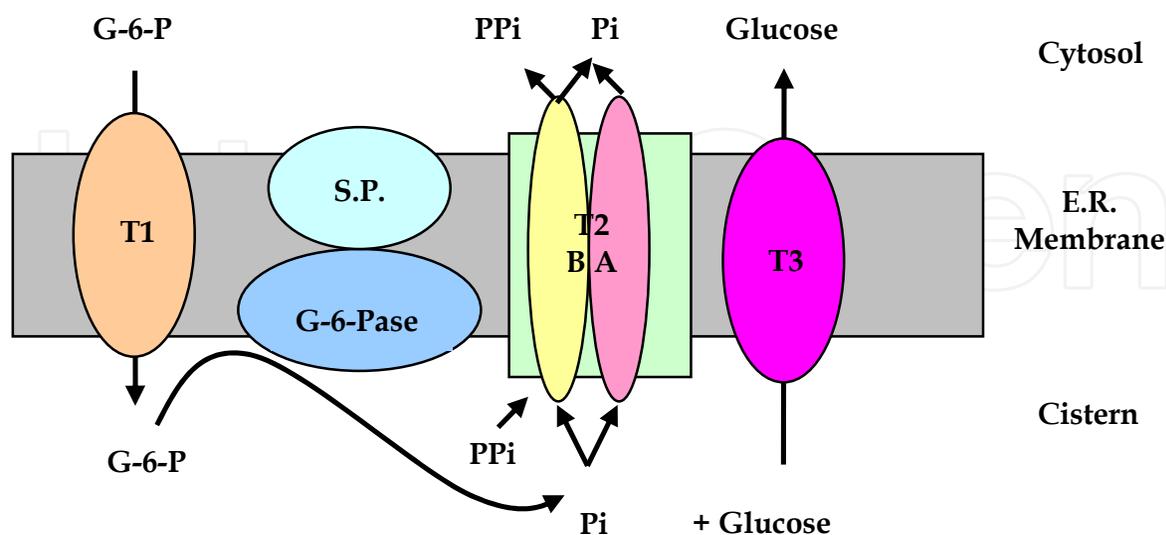


Fig. 2. Glucose intestinal absorption. It is a process that occurs in two steps, the first is mediated by SGLT1, which transports one molecule of glucose and 2 Na⁺ and is located at the apical membrane of the enterocyte. The second is carried out by the GLUT2 which transports glucose in favour of its gradient and is placed at the basolateral membrane of the cell. The participation of the Na⁺-K⁺ ATPase located on the basolateral membrane is also required to maintain the ionic gradients.

T1 is a highly specific transporter for glucose-6-phosphate, the substrate of the enzyme. The T2 is able to transport phosphate, pyrophosphate and carbamoylphosphate, and it has been suggested that comprises by 2 subunits. The glucose produced by the enzyme is transported by T3 also called GLUT 7. Finally it is thought there exists a stabilizing protein for the catalytic subunit. The glucose-6-phosphatase in addition to its hydrolytic activity, in conditions of high

glucose concentration and a phosphate donor such as pyrophosphate or carbamoylphosphate is able to synthesize glucose-6-phosphate (Foster & Nordlie, 2002).



1. Glucose-6-phosphate + H₂O -----> glucose + phosphate
2. Mannose-6-phosphate + H₂O -----> mannose + phosphate
3. Pyrophosphate + H₂O -----> 2 phosphates
4. Glucose + pyrophosphate -----> glucose6-phosphate + phosphate
5. Glucose + carbamoylphosphate -----> glucose-6-phosphate + NH₃ + CO₂

Fig. 3. Glucose-6-phosphatase model. Arion *et al.*, (1975) and Burchell & Waddell (1991) postulated the model of the catalytic subunit and transporters for the microsomal glucose-6-phosphatase. G-6-Pase: catalytic subunit; S.P: stabilizing protein; P_{PPi}: pyrophosphate; P_i: phosphate and E.R: endoplasmic reticulum. The glucose-6-phosphatase catalytic subunit is able to catalyze reactions 1-5.

The glucose transporters, GLUTs, (Joost & Thorens, 2001) mediate the facilitated transport of the glucose in and out of the different cells; of particular interest is the participation of the GLUT 4 which is located in skeletal and heart muscle and adipose tissue. The GLUT 4 is held in intracellular vesicles and by insulin action is translated to the plasma membrane and in consequence entry of glucose into those tissues increases thus lowering the glycaemia.

Drugs that inhibit the mechanism supplying glucose to the blood are considered antihyperglycaemic agents, on the other hand those that increase, directly or indirectly, the glucose entry to the tissues are considered hypoglycaemic agents. McCormack *et al.*, (2001) suggest that the enzyme glucose-6-phosphatase could be a potential target for antihyperglycaemic agents.

The microsomal fraction, which is enriched in endoplasmic reticulum, obtained by differential centrifugation is composed of intact and disrupted vesicles; the proportions of each can be measured assaying the glucose-6-phosphatase using mannose-6-phosphate as substrate, a molecule that is not translated by the T1 transporter (Arion *et al.*, 1976) in consequence all the mannose-6-phosphate hydrolyzed, by the microsomal fraction, is due to the activity of the catalytic subunit of the disrupted vesicles. The microsomal fraction

prepared without further treatment is called non-treated; the microsomes can be disrupted by the use of detergents, nitrogen cavitation, sonic disruption and more recently by the use of histones (Benedetti *et al.*, 2002). The term intact microsome is theoretical, and is estimated by subtracting from the enzyme activity of the non-treated microsomes that of the enzyme exhibited by the disrupted vesicles present in such preparations. Latency is a common characteristic of membranes bound enzymes, corresponding to the activity expressed only when the membrane are disrupted and is not present in the non-treated system. Generally it is calculated as the percentage of increase in activity due to the disruption of the vesicles.

Since time immemorial humans have been using plants for the empirical treatment of different illnesses, and in particular diabetes. In Venezuela, like many other tropical countries, the leaves of the *Bauhinia* species have been used by folk medicine in the empirical treatment of diabetes. The plants, belonging to the *Fabaceae* family (Hoyos 1978) are trees that can reach 10 m in height, with large well-developed evergreen leaves divided from the apex to 1/3 of its length and are hermaphrodite. The specie *Bauhinia megalandra* is characterized by having white flowers either single or in raceme with long and thin petals; the fruit is brown located in a compact pod. It is fast growing, long-lived and with deep roots (Figure 4).

The *Bauhinia megalandra* used on this work was always collected during the rainy season in the campus of the Universidad Central de Venezuela at Caracas and identified by Dr. Stefen Tillett of the Ovalles Herbarium of the Pharmacy Faculty of the Universidad Central de Venezuela.

2. Effects of *Bauhinia megalandra* leaf aqueous extract on gluconeogenesis and glucose-6-phosphatase

Due to good results reported for metabolic studies using precision-cut liver slices (McKee *et al.*, 1988; Dogterom, 1993), we studied the gluconeogenesis capacity of 200 μm thick liver slices prepared from 48 h fasted rats using a Krundieck Tissue Slicer (Alabama Research and Development, Alabama, USA), and incubated as described elsewhere (Krebs *et al.*, 1963), in Krebs-Ringer bicarbonate buffer supplemented with bovine serum albumin saturated with oleic acid and using lactate or fructose as gluconeogenic substrate (Gonzalez-Mujica *et al.*, 1998) in the absence (control) or presence an aqueous extract of *Bauhinia megalandra* leaves. As shown in Table 1, the gluconeogenic activity of the control liver slices was almost linear during 90 minutes and of the same magnitude with both substrates.

In the presence of the plant extract there was a drastic decrease in the gluconeogenic capacity of the liver slices, this being of higher magnitude when lactate was the gluconeogenic substrate in comparison with fructose, these results suggest that in the *Bauhinia megalandra* leaf extract compounds are present that inhibit the gluconeogenesis at a point further on than the entrance of both substrates.

The microsomal fraction used as source of the enzyme glucose-6-phosphatase was prepared as described elsewhere (Marcuci *et al.*, 1983), briefly: livers from overnight fasted rat were homogenized in 3 volumes of 0.32 M sucrose, 3 mM MgCl_2 , centrifuged at 20000 g for 20 min at 4° C, the supernatant was centrifuged at 105000 g for 1h at 4° C, the pellet constituting the microsomal fractions and was resuspended in 0.25 M sucrose 1 mM MgCl_2 5 mM HEPES pH 6.5 at a final protein concentration of 20 mg/mL and kept at -80° C until use. The entire microsomal fraction used in this work was at least constituted by 95 % intact vesicles.



Fig. 4. *Bauhinia megalandra*, flower and leaves.

Incubation Time (min)	Control	<i>Bauhinia megalandra</i>	
	Lactate or Fructose	Lactate	Fructose
30	31.1 ± 9.3	15.9 ± 7.3	18.1 ± 3.8
60	54.5 ± 17.2	11.6 ± 6.5	38.0 ± 6.0
90	90.2 ± 18.5	16.9 ± 8.8	34.0 ± 9.0

Table 1. Effects of *Bauhinia megalandra* leaf aqueous extract on hepatic gluconeogenesis. Liver slices from 48 hours fasted rats were incubated in Krebs-Ringer bicarbonate, supplemented with oleic acid saturated bovine serum albumin and in the presence of lactate or fructose as gluconeogenic substrate in the absence (control) or in the presence of 1 mg/mL of the *Bauhinia megalandra* leaf extract. At the indicated times, samples of the medium were withdrawn in order to measure glucose by the glucose oxidase-peroxidase method (Trinder 1969). The results are expressed in nmol of glucose produced/mg of liver dry weight and correspond to the means of 8-13 experiments ± standard deviation. The differences between control and plant extract treated were statistically significant at $p < 0.05$ for fructose and $p < 0.005$ for lactate.

The activity of the enzyme glucose-6-phosphatase was carried out following the method described by Burchell *et al.*, (1988) using glucose-6-phosphate or pyrophosphate as substrate in intact and disrupted (histone treated) hepatic microsomes. In brief, in a volume of 0.1 mL, 1-30 mM glucose-6-phosphate, 2 mM EDTA, 16 mM HEPES, pH: 6.5 and approximately 20 µg of microsomal proteins were present. In order to disrupt microsomes, 80 µg of histones were present in the incubation medium. The reaction was carried out at 30° C for 10 min without shaking and stopped by the addition of 0.9 mL of 0.28 % ammonium molybdate, 1.11 % SDS and 1.11 % ascorbic acid in 0.33 M sulphuric acid. The colour was developed at 47° C for 20 min and the absorbance was read at 820 nm. The assay was similar when pyrophosphate was

the substrate of the enzyme with the following differences: 0.5-5 mM pyrophosphate, 16 mM cacodilate, pH: 6.5, and the colour was developed at 30° C during 10 min.

Glucose-6-phosphate as substrate				
	Intact		Disrupted	
	V _{MAX}	K _M	V _{MAX}	K _M
Control	7.4 ± 0.9*	5.4 ± 1.1	9.8 ± 0.8	0.6 ± 0.1
10 µg/mL	6.2 ± 1.4	5.6 ± 0.7	9.8 ± 0.7	0.7 ± 0.2
20 µg/mL	5.1 ± 1.0*	7.5 ± 0.6	9.5 ± 0.3	0.7 ± 0.1

Pyrophosphate as substrate				
	Intact		Disrupted	
	V _{MAX}	K _M	V _{MAX}	K _M
Control	6.6 ± 1.4	1.3 ± 0.5	7.0 ± 0.9	0.4 ± 0.1
10 µg/mL	5.7 ± 1.6	1.0 ± 0.6	7.4 ± 0.8	0.4 ± 0.2
20 µg/mL	6.0 ± 1.5	1.2 ± 0.4	6.6 ± 1.3	0.3 ± 0.3

Table 2. Kinetic parameters of hepatic microsomal glucose-6-phosphatase in the absence or presence of the aqueous extract of *Bauhinia megalandra* leaves. The activity of the hepatic microsomal glucose-6-phosphatase was assayed using glucose-6-phosphate or pyrophosphate as substrate in the absence (control) or in the presence of the indicated concentration of the aqueous extract of *Bauhinia megalandra* leaves. The kinetic parameters were calculated from the Michaelis and Menten graphs using the Enzfitter program (Leatherbarrow 1987) and the V_{MAX} for glucose-6-phosphatase activity is expressed in µmol of phosphate liberated/h x mg of microsomal proteins and the V_{MAX} for the pyrophosphatase activity is expressed in µmol of pyrophosphate hydrolyzed/h x mg of microsomal proteins; for both the K_M is expressed in mM. The experiments were performed using the liver of 3-5 fasted (24 h) rats, and each point represents the mean of 3-4 experiments ± standard deviation. * Means differences statistically significant at p < 0.05.

As shown in Table 2, when glucose-6-phosphate was the substrate of the glucose-6-phosphatase of intact microsomes, the plant extract decreased in a statistically significant way the V_{MAX} with a moderate increase of the K_M without affecting the enzyme of disrupted microsomes.

The plant extract was without effect on the glucose-6-phosphatase activity in intact or disrupted microsomes when pyrophosphate was the substrate (Table 2). These results suggest that the compounds present in the *Bauhinia megalandra* aqueous extract inhibit one of the transporters of the glucose-6-phosphatase system, probably T1 (Gonzalez-Mujica et al., 1998). The inhibition of the hepatic gluconeogenesis could be a consequence of the inhibition of the glucose-6-phosphate transporter (T1).

The glucose-6-phosphatase activity of non-treated microsomes, when assayed with 15 mM glucose-6-phosphate as substrate, was linear during 30 min. (Figure 5); in the other hand, in the presence of 20 µg/mL of the plant extract the enzyme activity was linear just up to 20 min, but all the values were lower than the control, after that an inflexion occurred and the slope of the line declined (Figure 5), suggesting that some compound(s) present in the *Bauhinia megalandra* leaf extract inhibit other transporter of the glucose-6-phosphatase

system, different than T1, and in consequence the enzyme products accumulated in the microsomal cistern leading to inhibition of the catalytic subunit. Due to the fact that the plant extract does not affect the kinetic parameters of the glucose-6-phosphatase using pyrophosphate as substrate, there is no effect on the pyrophosphate transporter (T2), it is possible to suggest that the inhibition occurs at the glucose transporter (T3).

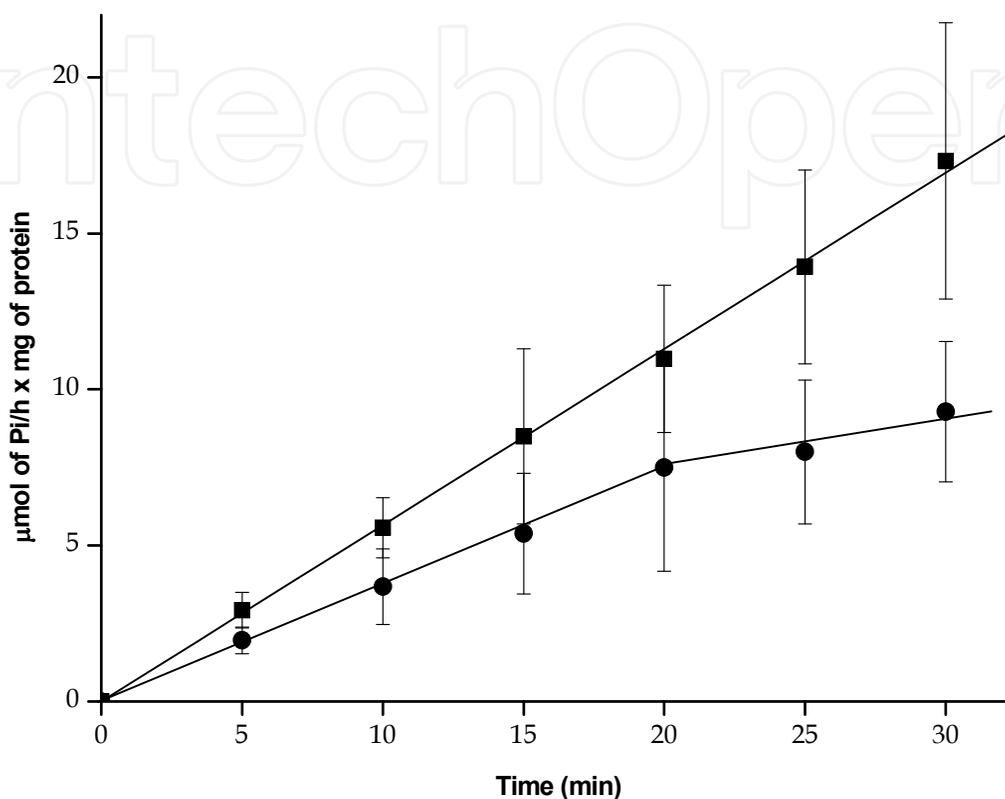


Fig. 5. Effects of *Bauhinia megalandra* leaf extract on the time course of hepatic microsomal glucose-6-phosphatase. The activity of the glucose-6-phosphatase was measured in the absence (■) or in the presence (●) of 20 µg/mL of *Bauhinia megalandra* leaf extract at 15 mM glucose-6-phosphate; each point represents the mean of 5 experiments and the vertical bars correspond to standard deviation. The enzyme activity is expressed in µmol of phosphate (Pi) released /hour x mg of protein. All the differences observed between control and extract treated microsomes were statistically significant at $p < 0.05$ except that seen at 20 min.

3. Isolation and identification of flavonoids from *Bauhinia megalandra* leaves. Characterization of their effects on hepatic microsomal glucose-6-phosphatase and gluconeogenesis

We fractionated the methanol extract of fresh *Bauhinia megalandra* leaves using different solvents (methanol : water 1:1; ethyl acetate : acetone 8:2) and column chromatography on sephadex LH-20 and RP-18 (Estrada et al., 2005) to obtain the following flavonoids: astilbin (1), quercetin 3-O- α -rhamnoside (2), kaempferol 3-O- α -rhamnoside (3), quercetin 3-O- α -arabinoside (4), quercetin 3-O- α -(2''-galloyl)rhamnoside (5), kaempferol 3-O- α -(2''-galloyl)rhamnoside (6), quercetin (7) and kaempferol (8). The identification of the above mentioned flavonoids was established by comparing their ^1H and ^{13}C

NMR chemical shifts and proton coupling constants in DMSO-d₆ with those reported (Agrawal, 1989; Markham and Geiger, 1994; De Brito et al., 1995; Méndez et al., 1995; Bilia et al., 1996) and the structure of compounds **5** and **6** is shown in Figure 6. To the best of our knowledge, this was the first report of the isolation of kaempferol 3-O- α -(2''-galloyl)rhamnoside from nature.

The effect of the isolated flavonoids on the activity of the hepatic glucose-6-phosphatase was carried out using intact and disrupted microsomes with glucose-6-phosphate as substrate as indicated above.

The inhibition of the enzyme by the flavonoids isolated from *Bauhinia megalandra* leaves in intact microsomes, but not in disrupted ones (Table 3), is a clear indication that they interact with the glucose-6-phosphate transporter (T1) of the glucose-6-phosphatase system with no effect on the catalytic subunit. It is interesting to point out that the flavonoids tested behave in a similar way to phlorizin (Table 3) only inhibiting the T1 transporter (Arion *et al.*, 1980).

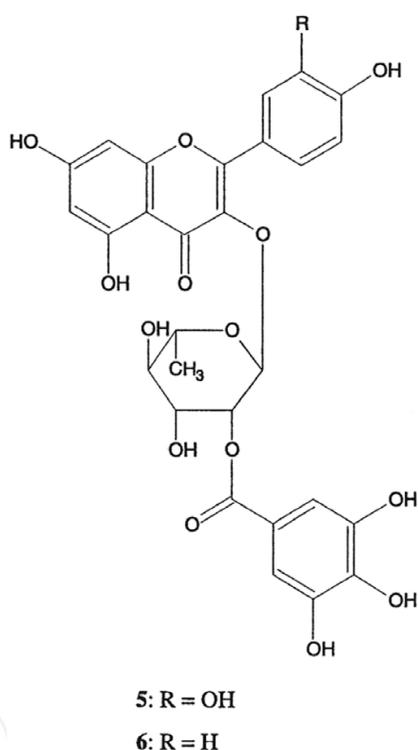


Fig. 6. Structure of quercetin 3-O- α -(2''-galloyl)rhamnoside (**5**) and kaempferol 3-O- α -(2''-galloyl)rhamnoside (**6**)

As can be seen in Table 3, the aglycones, quercetin (**7**) and kaempferol (**8**), showed the lowest inhibitory effect on the enzyme and the percentage inhibition increased with the addition of the polar group rhamnose or arabinose as in the case of compounds **1**, **2**, **3** and **4**, which exhibited moderate effects. The flavanone glycoside evaluated (**1**) showed a similar behavior to compounds **2** and **3**, but to a smaller extent; this could be attributed to the saturation of the double bond in the C-ring of the flavonoid nucleus. The strongest inhibitory effect was observed when the galloyl moiety was present (**5** and **6**). This observation suggests the possibility of a particular interaction between this polyphenolic residue and the glucose-6-phosphate transporter (T1), because the complex formation

between proteins and tannins in which gallic acid is usually one of the main components, is well known (Haslam *et al.*, 1989).

Compound	Intact microsomes			Disrupted microsomes	
	Activity	%	IC ₅₀	Activity	%
Control	2.77 ± 0.28			5.66 ± 0.45	
Astilbin (1)	2.23 ± 0.42 ^a	-19.5	9.59 ± 27	5.80 ± 0.43	+2.5
Quercetin 3-O- α -rhamnoside (2)	1.95 ± 0.36 ^b	-29.6	246 ± 5	5.42 ± 0.53	-4.2
Kaempferol 3-O- α -rhamnoside (3)	2.04 ± 0.25 ^b	-26.4	186 ± 4	5.84 ± 0.46	+3.2
Quercetin 3-O- α -arabinoside (4)	2.04 ± 0.23 ^b	-26.4	1069 ± 31	5.77 ± 0.18	+3.0
Quercetin 3-O- α -(2''-galloyl)rhamnoside (5)	1.02 ± 0.22 ^c	-63.2	27 ± 1	6.43 ± 0.52 ^a	+13.6
Kaempferol 3-O- α -(2''-galloyl)rhamnoside (6)	0.93 ± 0.27 ^c	-66.4	31 ± 2	6.61 ± 0.41 ^b	+16.8
Quercetin (7)	2.43 ± 0.05 ^c	-12.3	1330 ± 39	5.81 ± 0.52	+2.7
Kaempferol (8)	2.71 ± 0.49	-2.2	555 ± 6	5.98 ± 0.16	+5.7
Phlorizin	2.00 ± 0.11 ^b	-27.8	4.66 ± 19	5.71 ± 0.40	+0.9

Table 3. Effects of flavonoids isolated from *Bauhinia megalandra* on glucose-6-phosphatase. The glucose-6-phosphatase was assayed using 5 mM glucose-6-phosphate as substrate in intact and disrupted microsomes in the absence (control) or presence of 50 μ M of each of the flavonoids isolated from *Bauhinia megalandra* leaves or 50 μ M phlorizin. The enzyme activity is expressed as μ mol of phosphate released /h x mg of microsomal protein and each value represents the means of 5-9 experiments \pm standard deviation. The percentage inhibition (-) or activation (+) was also calculated.

The IC₅₀ was carried out following Arion *et al.*, (1998) using intact microsomes with 1mM glucose-6-phosphate in the presence of increasing concentrations (including values below and above the IC₅₀) of the isolated flavonoids; the result are the average of 3 experiments \pm standard deviation, and expressed in μ M.

The increase in the glucose-6-phosphatase inhibition capacity with increase in the polarity of the flavonoids suggests a specific interaction with the glucose-6-phosphate transporter of the enzyme system because it cannot be related to a higher solubility in the endoplasmic reticulum membrane. Also, the interaction seems to be specific view of the fact that none of the flavonoids was able to inhibit the catalytic subunit of the glucose-6-phosphatase system (Table 3). The greater enzyme inhibition exerted by quercetin (7) in comparison to kaempferol (8)(Table 3) could be explained by the fact that the former has one additional vicinal hydroxyl group on the B-ring with respect to the latter. The difference in polarity between the aglycones is overcome by the presence of the carbohydrate and galloyl moieties, and in consequence, the inhibitory effect was also affected.

The compounds 5 and 6 showed the lowest IC₅₀ value and were very close to each other (Table 3). The lack of gallic acid in compounds 2 and 3 increased the IC₅₀ value 9 and 6 times respectively, with respect to compounds 5 and 6. The aglycones, compounds 7 and 8, exhibited high IC₅₀ values, which are 49 and 18 times higher than those obtained with compounds 5 and 6, respectively (Table 3). Compounds 1 and 4, although glycosylated, showed high IC₅₀ values, comparable to that observed with the aglycone quercetin (7). To

our knowledge, this has been the first report of the IC_{50} for phlorizin and glucose-6-phosphatase. There is a good agreement between the percentages of glucose-6-phosphatase inhibition and the IC_{50} produced by the flavonoids tested. The sugar bound to the flavonoid seems to be important due to the fact that substitution of rhamnose by arabinose increases by more than 4 times the IC_{50} (Table 3). The reduction of the double bond of ring C, in astilbin, also increases the IC_{50} by almost 4 times (Table 3).

All the flavonoid isolated, but one (2) produce activation of the glucose-6-phosphatase catalytic subunit, however, only compounds 5 and 6 (Table 3) do so to an appreciable magnitude and this could be due to modifications in the microsomal membranes that expose the active center of the enzyme, as a consequence of the presence of the tannic flavonoids.

Due to the fact, that quercetin 3-O- α -(2''-galloyl)rhamnoside (5) exerts high inhibition of the T1 transporter of the glucose-6-phosphatase system and showed the lowest IC_{50} of all the flavonoids tested, we studied the effects of that compound on the kinetic parameters of the enzyme using glucose-6-phosphate or pyrophosphate as substrate. The enzyme assay was carried out as described above. The enzyme assays, with both substrates were carried out in the absence (control) or presence of 15 μ M quercetin 3-O- α -(2''-galloyl)rhamnoside (5).

Glucose-6-phosphate as substrate

	Intact microsomes		Disrupted microsomes	
	V_{MAX}	K_M	V_{MAX}	K_M
Control	7.59 \pm 0.91	4.78 \pm 1.10	11.57 \pm 1.55	0.79 \pm 0.27
Quercetin 3-O- α -(2''-galloyl)rhamnoside (5)	7.36 \pm 1.79	7.44 \pm 0.42*	10.47 \pm 1.45	0.57 \pm 0.17

Pyrophosphate as substrate

	Intact microsomes		Disrupted microsomes	
	V_{MAX}	K_M	V_{MAX}	K_M
Control	7.61 \pm 2.45	2.28 \pm 1.35	8.40 \pm 1.73	0.40 \pm 0.11
Quercetin 3-O- α -(2''-galloyl)rhamnoside (5)	6.72 \pm 1.33	1.87 \pm 0.41	7.58 \pm 0.73	0.24 \pm 0.07

Table 4. Effects of quercetin 3-O- α -(2''-galloyl)rhamnoside (5) on the kinetic parameters of the glucose-6-phosphatase. Microsomal glucose-6-phosphatase was assayed using glucose-6-phosphate (1-30 mM) or pyrophosphate (0.5-5 mM) as substrate in the absence (control) or presence of 15 μ M quercetin 3-O- α -(2''-galloyl)rhamnoside (5) as described in the text. The kinetic parameters were calculated from the Michaelis and Menten graph using the Enzfitter program. The V_{MAX} is expressed as μ mol of phosphate released /h/mg proteins when glucose-6-phosphatase was the substrate and in μ mol of pyrophosphate hydrolysed /h/mg proteins and the K_M in mM. Each value represents the means of 3-5 experiments \pm standard deviation and in each experiment, the livers of 3-4 rats were used. * Means differences statistically significant at $p < 0.005$.

Table 4 show the effects of quercetin 3-O- α -(2''-galloyl)rhamnoside (5) on the kinetic parameters of the microsomal glucose-6-phosphatase using glucose-6-phosphate or pyrophosphate as substrate. In controls using glucose-6-phosphate as substrate, when the microsomes were disrupted by the presence of histones, there was an increase in the V_{MAX} with a latency of 34.4% and the K_M was 1/6 of the value observed in the intact system; these results are in good agreement with those reported elsewhere (Blair and Burchell, 1988;

Gonzalez-Mujica *et al.*, 1993, 1998). In intact microsomes the presence of quercetin 3-*O*- α -(2''-galloyl)rhamnoside (**5**) increased the K_M for glucose-6-phosphate by nearly 56%, a change that was statistically significant at $p < 0.005$. On the other hand, there were no changes in the V_{MAX} of intact microsomes neither in the V_{MAX} nor in the K_M of the disrupted system. When pyrophosphate was the substrate, the activity showed a lower latency (9.4%) and a similar decrease of the K_M (1/6) compared with that shown when glucose-6-phosphate was the substrate and the microsomes were disrupted, results that are similar to those reported by others (Blair and Burchell, 1988; Gonzalez-Mujica *et al.*, 1993, 1998). The kinetic parameters of the enzyme using pyrophosphate as substrate in intact and disrupted microsomes were not altered by the inclusion of the flavonol.

From the above results: increase of the K_M for glucose-6-phosphate in intact microsomes without affecting the other kinetic parameter in intact or disrupted microsomes using glucose-6-phosphate or pyrophosphate as substrates, is a clear indication that quercetin 3-*O*- α -(2''-galloyl)rhamnoside (**5**) behaves as a competitive inhibitor of the T1 transporter without altering the catalytic subunit nor the other transporters of the glucose-6-phosphatase system. It is interesting to point out that a synthetic derivative of chlorogenic acid, a compound without any structural relationship with glucose-6-phosphate has been reported as a competitive inhibitor of T1 (Arion *et al.*, 1998)

Hepatic gluconeogenesis was studied using 48 h fasted rat liver slices of 200 μm thick incubated in Krebs-Ringer bicarbonate buffer, as described above, in the absence (control) or the presence of 30 μM quercetin 3-*O*- α -(2''-galloyl)rhamnoside (**5**). The incubations were carried out with 10 mM lactate as gluconeogenic substrate, at 37 °C, under an $\text{O}_2:\text{CO}_2$ (95:5) atmosphere, with continuous shaking in an orbital bath (60 cycles/min), for a total time of 90 min. Samples for glucose determination were taken every 30 min. At the end, the slices were dehydrated with acetone and dried at 70 °C for 24 h and the dry weight of the slices was determined. Glucose determination was made by the glucose oxidase method (Trinder, 1969) and the gluconeogenic activity was expressed as nmol of glucose produced/mg liver dry weight.

The gluconeogenic capacity of the rat liver slices in the absence (control) and presence of quercetin 3-*O*- α -(2''-galloyl)rhamnoside (**5**) is shown in Fig. 7. The glucose production by the control liver slices was almost linear with time and similar to that reported earlier (Gonzalez-Mujica *et al.*, 1998). Quercetin 3-*O*- α -(2''-galloyl)rhamnoside (**5**) drastically reduced the gluconeogenic capacity of the liver slices at all the times studied: at 30 min the inhibition was approximately 85%; the inhibition was nearly 50% at 60 min. and 41% at 90 min.

The inhibition of the T1, the glucose-6-phosphate transporter, of the hepatic microsomal glucose-6-phosphatase system by quercetin 3-*O*- α -(2''-galloyl)rhamnoside (**5**), might explain the inhibition of the gluconeogenic capacity of the rat liver slices in the presence of the flavonol and, in turn, could reduce the hepatic glucose production; this antihyperglycaemic effect serve to decrease the blood glucose level in diabetic patients.

4. Effects of *Bauhinia megalandra* leaf aqueous extract on glycogenolysis

An aqueous extract of *Bauhinia megalandra* leaves was administered by a gastric tube to rats anesthetized with sodium pentobarbital (30 mg/Kg body weight), 1 h later a blood sample was taken from the tail and 0.15 mg/Kg body weight of epinephrine (Cori and Cori 1928) was

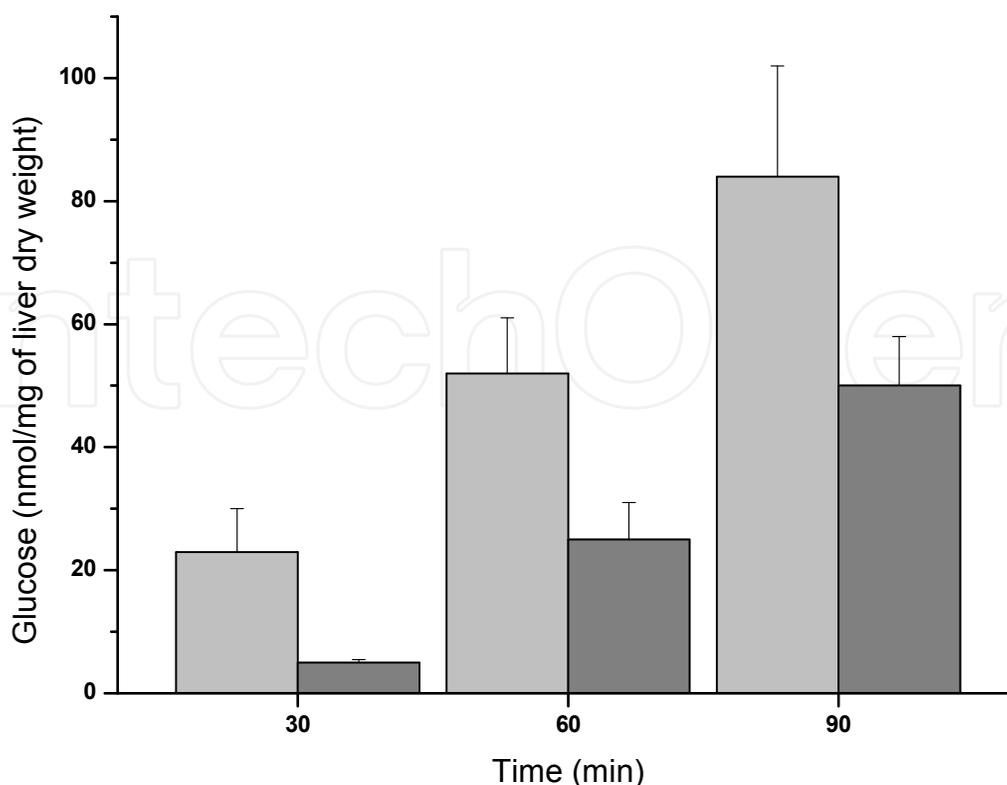


Fig. 7. Effects of quercetin 3-O-α-(2''-galloyl)rhamnoside (**5**) on hepatic gluconeogenesis. Liver slices from 48 h fasted rats were incubated in 4 mL Krebs-Ringer bicarbonate buffer supplemented with oleate saturated albumin using lactate as gluconeogenic substrate, in the absence (light grey) or presence of 30 μM of Quercetin 3-O-α-(2''-galloyl)rhamnoside (**5**) (dark grey). At the indicated times, medium samples were withdrawn in order to measure glucose by the glucose oxidase method. The results are the average of 7–9 separate experiments ± standard deviation. All the differences between the gluconeogenesis of controls and quercetin 3-O-α-(2''-galloyl)rhamnoside (**5**) treated liver slices were statistically significant at $p < 0.005$.

given by intraperitoneal injection, and afterwards blood samples were taken, as before, every 30 min for 2 h; glucose was estimated by the glucose oxidase-peroxidase method (Trinder 1969).

As shown in Figure 8, the administration of epinephrine substantially increases the glycaemia with a maximum at 60 min (over 80 % increases). When the plant extract was given before the catecholamine there was an important reduction of the hyperglycaemia induced by epinephrine (over 24 % reduction), been statistically significant at 60 min (Fernández- Peña, *et al.*, 2008). These results suggest the intestinal absorption of compounds present in the *Bauhinia megalandra* leaves, which reach the liver via the portal vein and are capable of inhibiting the glycogenolysis direct or indirectly.

In order to establish the mechanism of action of such compounds, we incubated fed rats liver slices in the same way as described before (Figure 7) with just buffer alone (control) or in the presence of epinephrine (3.3 μM) or dibutyryl cyclic AMP (10 nM) or the plant extract (3.44 mg/L) or the combinations of epinephrine and the vegetal extract or dibutyryl cyclic AMP and the plant extract.

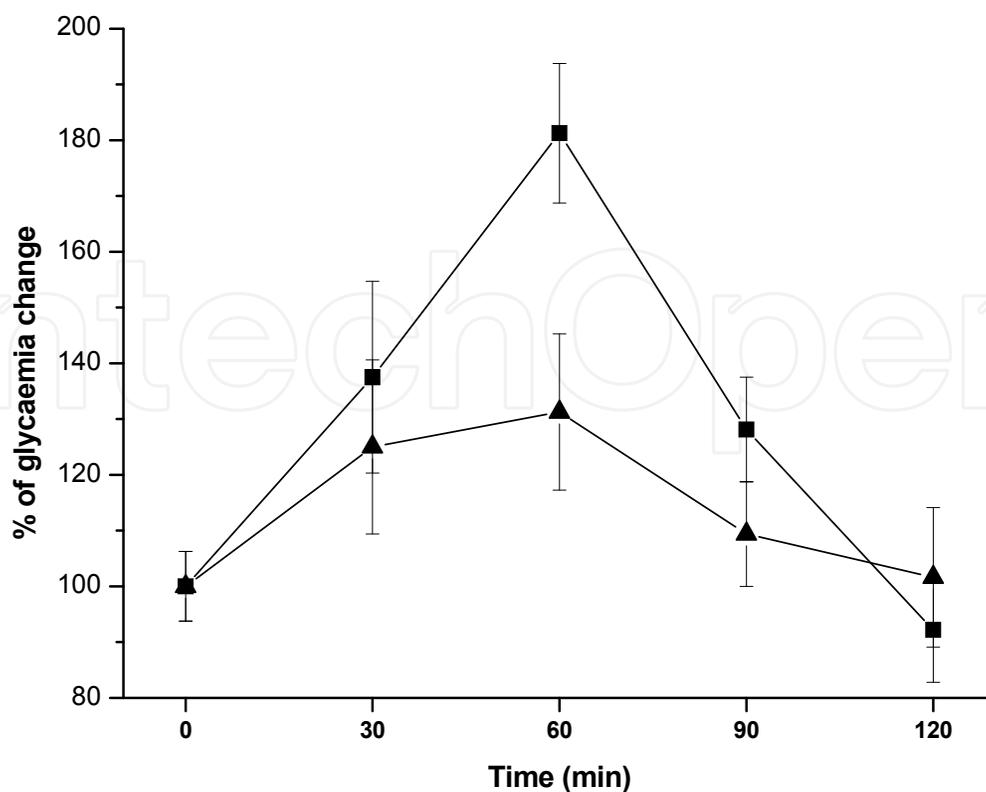


Fig. 8. Effects of *Bauhinia megalandra* aqueous extract on the hyperglycaemia induced by epinephrine. Male rats were anaesthetized (sodium pentobarbital 30 mg/Kg body weight) before the administration by a gastric tube of water (control -■-) or 3.44 g/Kg of body weight of the plant extract (-▲-). One h later the animals receive by intraperitoneal injection 0.15 mg/Kg body weight of epinephrine and the glycaemia was measured every 30 min for 2 h. The basal glycaemia of fed rats (100 %) was 7.2 ± 0.5 mM. The values represent the means \pm standard deviation of 5 - 6 experiments and in each 3 rats were used. At 60 min the differences between control and experimental was statistically significant a $p < 0.001$.

As shown in Figure 9 the amount of glucose released by the rat liver slices was increased by the presence of the catecholamine, being 60 and 54% of the control values at 60 and 90 min respectively. When epinephrine and the plant extract were present simultaneously, the glucose production by the liver slices was almost parallel with, but significantly lower than that observed with the hormone alone (Figure 9), being 35; 25 and 24% of the control values at 30; 60 and 90 min respectively. It is interesting to point out, that at 30 min the glucose production in the joint presence of epinephrine and the plant extract was 35% lower than that observed in the control.

As shown in Figure 10, the presence of dibutyryl cAMP produced an increase in the glucose release by the liver slices (Fernández-Peña, *et al.*, 2008) at all times studied, being more evident at 60 min, when it was 2.6 times the control value.

In the joint presence of the cyclic nucleotide and the plant extract there was a drastic reduction of the dibutyryl cAMP effect, at 60 min the glucose production was decreased by almost 50 %. The results presented (Figures 8; 9 and 10) strongly suggest that in *Bauhinia megalandra* leaf extract there are compounds that inhibit hepatic glycogenolysis. We also presented evidences that the plant extract inhibits the gluconeogenesis (Table 1 and Figure 7),

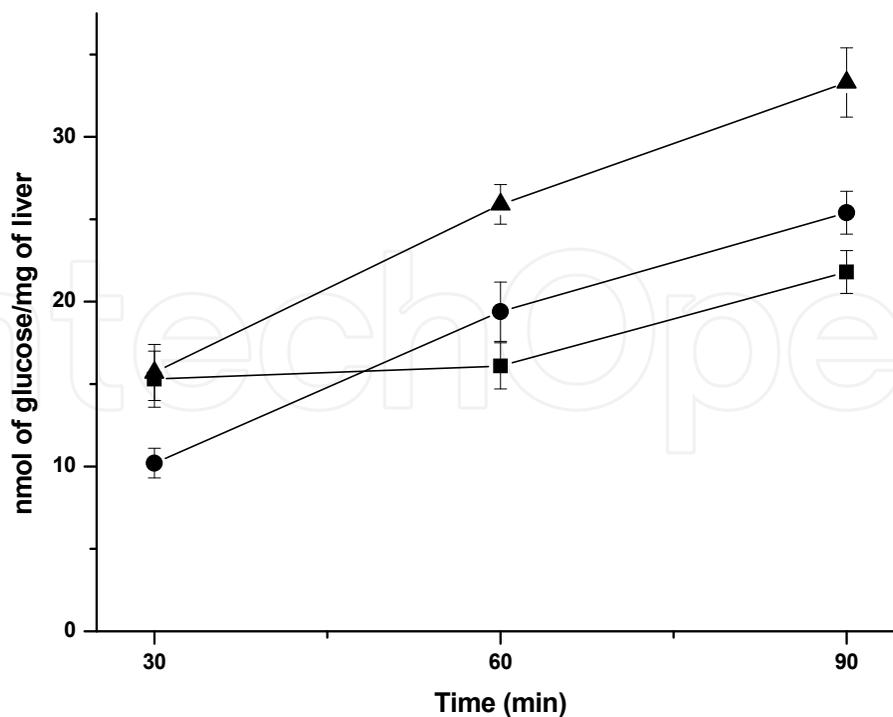


Fig. 9. Effects of epinephrine and of *Bauhinia megalandra* leaf extract on hepatic glycogenolysis. Fed rats liver slices were incubated in 4 mL of Krebs-Ringer bicarbonate buffer pH 7.4 in an atmosphere of O₂/CO₂ 95/5 without additions for control (-■-), or in the presence of 3.3 μM epinephrine (-▲-), or the combination of 3.3 μM epinephrine and 13.8 μg of the plant extract (-●-). At the indicated times glucose was measured in samples of the incubation medium as before. The values are the means ± standard deviations of 5 – 6 experiments, in each 3 rats were used. At 60 and 90 min, the differences between epinephrine and control and the combination of epinephrine and the plant extract were statistically significant at $p < 0.001$.

and due to the fact that the only enzyme common to both metabolic pathways, glycogenolysis and gluconeogenesis is the glucose-6-phosphatase in consequence this enzyme should be the target of the compounds present in *Bauhinia megalandra* leaf extract. Furthermore, the plant extract and the flavonoids isolated from it, inhibit the T1, the glucose-6-phosphate transporter of the hepatic microsomal glucose-6-phosphatase system. The above result, strongly suggest that the flavonoids present in *Bauhinia megalandra* leaf extract inhibit hepatic glycogenolysis and gluconeogenesis because of inhibition of the glucose-6-phosphatase and in consequence there is a reduction in the glucose production by the liver. These flavonoids might be useful in the treatment of non insulin dependent diabetes because of its antihyperglycaemic activity.

5. Effects of *Bauhinia megalandra* aqueous extract and Kaempferol 3-O- α -rhamnoside on glucose intestinal absorption

The glucose intestinal absorption was measured by the method described by Gonzalez-Mujica *et al.*, (2003). In rats anaesthetized with sodium pentobarbital (60 mg/Kg body weight), the intestine was exposed and divided *in situ* into 4 cm segments by ligatures; 1 mL of 10 mM glucose, 0.9 % NaCl alone or with the plant extract (1.14-9.10 mg) or extract

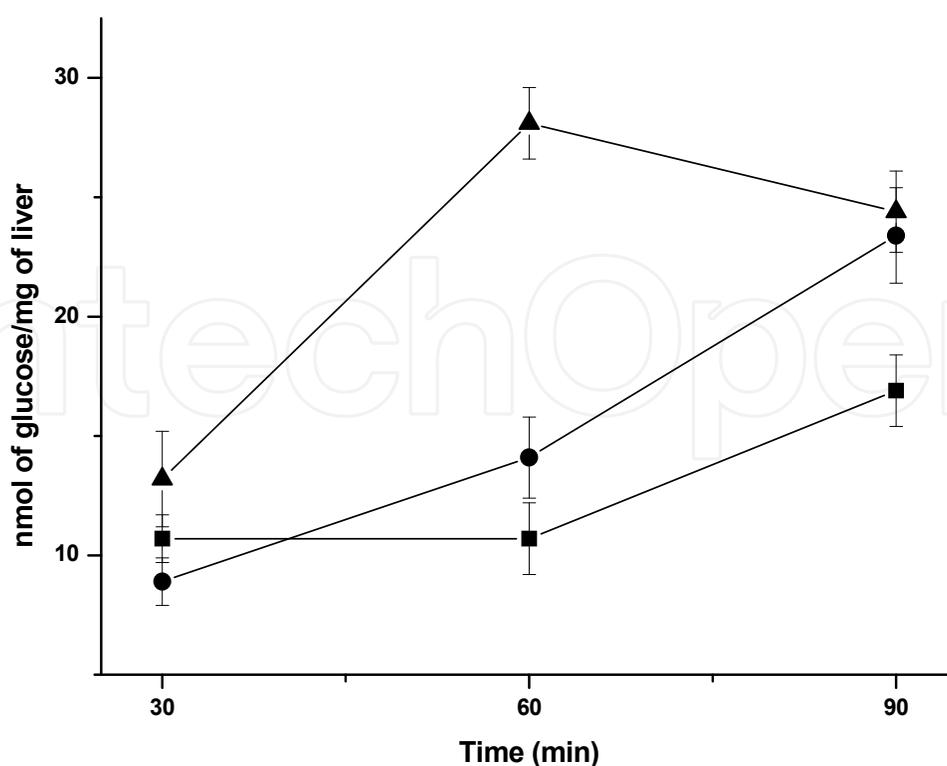


Fig. 10. Effects of dibutyryl cAMP and of *Bauhinia megalandra* leaf extract on hepatic glycogenolysis. All conditions were the same as those indicated in Figure 9, without addition for control (-■-), or in the presence of 10 nM of dibutyryl cAMP(-▲-), or the combination of 10 nM of dibutyryl cAMP and 13.8 µg of the plant extract (-●-). At 60 min the differences observed between the values obtained in the presence of the cyclic nucleotide alone and those in controls and in the presence of the mixture of dibutyryl cAMP and the plant extract were statistically significant at $p < 0.001$.

fractions (10 mg/mL) or pure compounds (5 mM), as indicated in the figures, was injected. Phlorizin, a well known inhibitor of sodium-glucose cotransporter 1 (SGLT1)(Panayatova-Heiermann, *et al.*, 1995), at a concentration of 0.1 mM was used as a positive control. After 30 min the intestinal segment contents were recovered without significant change in volume, and the glucose was measured (Trinder, 1969).

As shown in Table 5, the aqueous extract of *Bauhinia megalandra* leaves inhibits the glucose intestinal absorption in a concentration- dependant way. When the plant extract (4.55 mg) was injected together with 0.1 mM phlorizin, an additive inhibitor effect was observed. The concentration dependent inhibition exerted by the plant extract indicates a specific action and due to the fact that phlorizin is a known inhibitor of the sodium-glucose cotransporter 1 (Panayatova-Heiermann, *et al.*, 1995), the observed additive effect of *Bauhinia megalandra* leaf extract and phlorizin suggest that in the plant there are compounds that inhibit that cotransporter.

Vesicles from enterocyte apical membrane were prepared by the Ca^{++} precipitation method described by Kessler *et al.*, (1978). The intestinal membrane vesicles obtained contained maltase measured as described by SaMoita, *et al.*, (1989) in a ratio vesicles/homogenate 9.84; a small amount of $\text{Na}^{+}/\text{K}^{+}$ ATPase sensitive to ouabain, estimated following Del Castillo & Robinson (1982) in a ratio vesicles/homogenate 0.42; succinate-cytochrome c reductase

<i>B megalandra</i> Extract	A	B	
	nmol of glucose absorbed	Addition	nmol of glucose absorbed
0 mg	774.2 ± 34.4*	0	773.6 ± 70.3
1.14 mg	602.2 ± 103.2**	4.55 mg <i>B megalandra</i> extract	348.9 ± 79.1**
2.28 mg	464.5 ± 60.2**		
4.55 mg	395.7 ± 51.6**	0.1 mM phlorizin	392.1 ± 80.9**
6.5 mg	223.7 ± 36.1**	4.55 mg <i>B megalandra</i> extract + 0.1 mM phlorizin	87.9 ± 35.2**
9.10 mg	141.1 ± 25.8**		

Table 5. Effects of *Bauhinia megalandra* extract on glucose intestinal absorption. In each of four consecutive intestinal segments were injected, *in situ*, 1mL of 10 mM glucose, 0.14% NaCl alone (0 mg) or in the presence of increasing amounts of *Bauhinia megalandra* leaf extract (A); or in the presence of 4.55 mg of the plant extract, or 0.1 mM phlorizin or the combination of 4.55 mg of plant extract and 0.1 mM phlorizin (B). After 30 min the glucose was measured in the intestinal segments content, expressed in nmol of glucose absorbed in 30 min and each value corresponds to the mean ± standard deviation of eight experiments. * Means $p < 0.005$ and ** means $p < 0.0005$.

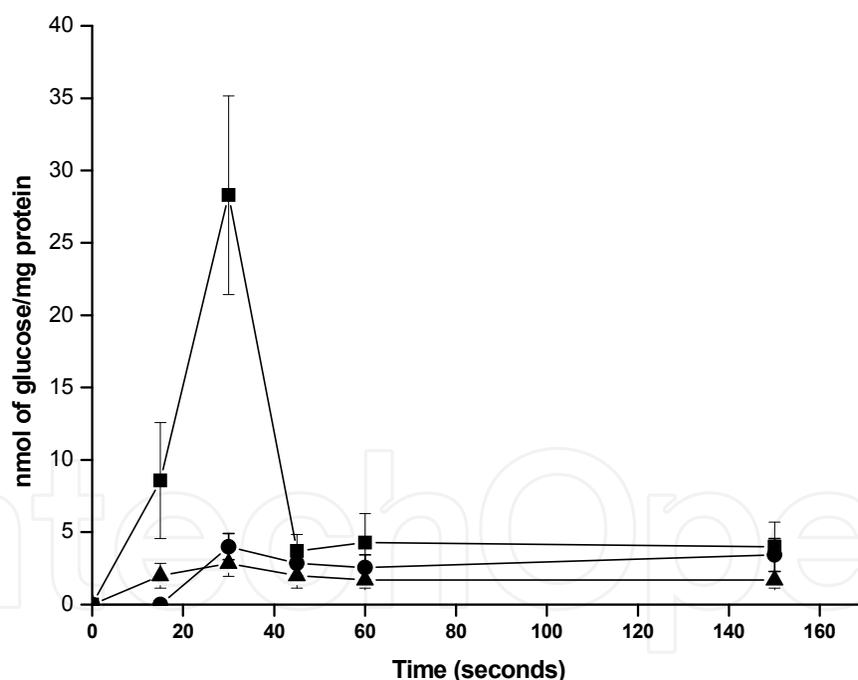


Fig. 11. Effects of *Bauhinia megalandra* on ^{14}C -glucose uptake by enterocyte brush border membrane vesicles. Rat enterocyte brush border membrane vesicles internally filled with 100 mM KCl were incubated with 2 mM ^{14}C -glucose (0.1 μCi) and 100 mM NaCl for the control (-■-) or with the addition of 4.55 μg of *Bauhinia megalandra* aqueous extract (-▲-) or with the addition of 1 mM phlorizin (-●-). At the indicated times the reaction was stopped by the fast filtration method (Hopfer, *et al.*, 1973) and the radioactivity was measured. The values are expressed in nmol of glucose uptake/ mg of vesicles protein and correspond to the mean ± standard deviation of 9 experiments with at least 2 rats in each one.

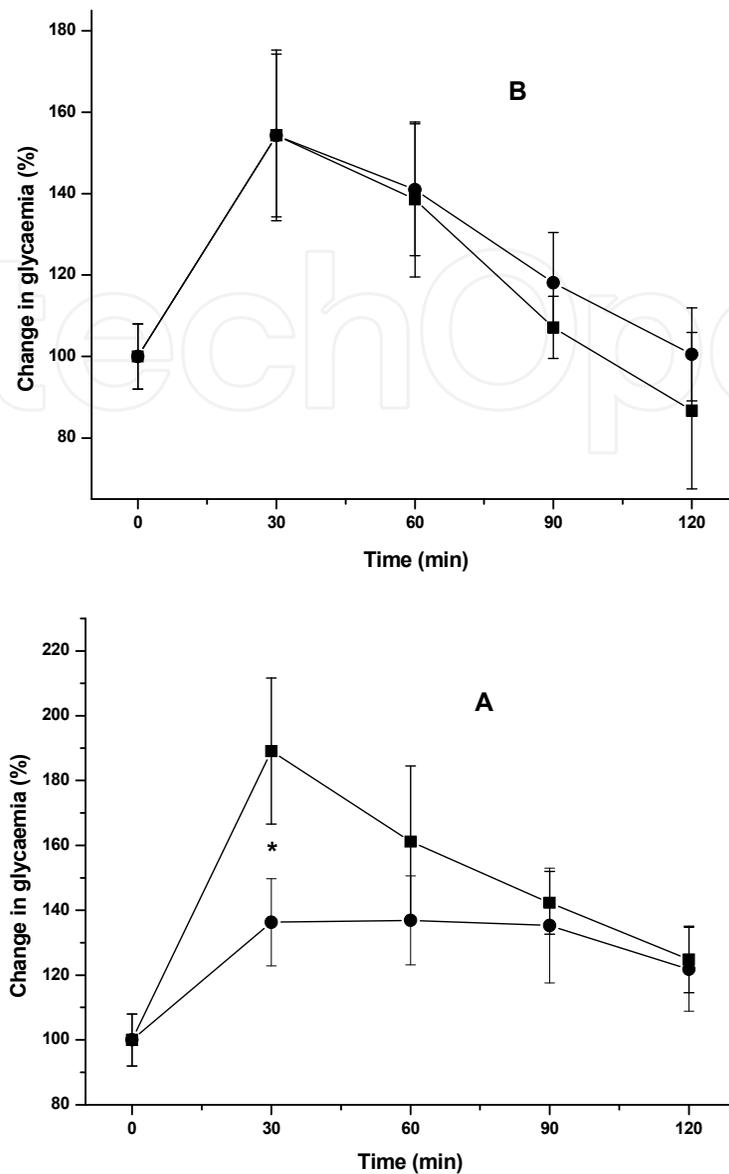
quantified by the method of Green, *et al.*, (1955) in a ratio vesicles/homogenate 0.36 and β -glucuronidase measured as described by Bergmeyer (1965) in a ratio vesicles/homogenate 0.09. Electronmicroscopy showed vesicles with a single membrane without electron dense material inside. These results are in good agreement with those reported by Kessler *et al.*, (1978) and indicate that the vesicles fraction obtained was enriched with brush border membrane, with low contamination with other cellular membrane. The ^{14}C -glucose uptake by the intestinal brush border membrane vesicles, obtained as described above, was performed by the fast filtration method (Hopfer, *et al.*, 1973) in the absence or presence of the *Bauhinia megalandra* leaves extract or 1mM phlorizin.

As shown in Figure 11 the ^{14}C -glucose uptake by intestinal brush border membrane vesicles exhibits a peak at 30 s followed by a plateau between 45 and 150 s, a result very similar to that previously published (Kessler *et al.*, 1978). When the intestinal brush border membrane vesicles were incubated in the presence of 4.55 μg of the plant extract or 1 mM phlorizin there was complete inhibition of the 30 s ^{14}C -glucose peak uptake. These results clearly indicate that in the plant extract there are compounds that inhibit the sodium-glucose cotransporter 1 and in consequence are similar to those reported for green tea polyphenols (Kobayashi, *et al.*, 2000) and soyabean isoflavone (Vedavanam, *et al.*, 1999).

As shown in Figure 12-A, the simultaneous administration of *Bauhinia megalandra* leaf extract (260 mg) together with glucose (1g/Kg body weight) by gastric catheter produced an almost flat glucose tolerance, with a decrease in nearly 28 % in the 30 min peak which was statistically significant in comparison with the control receiving water instead of the plant extract. On the other hand, when the *Bauhinia megalandra* leaf extract (260 mg) was administrated orally and the glucose (1g/Kg body weight) by subcutaneous injection (Figure 12-B) there was no difference with the control that received water instead of the plant extract. The flat oral glucose tolerance (Figure 12-A) in comparison with the normal subcutaneous glucose tolerance (Figure 12-B) when the glucose was administered simultaneously with plant extract, strongly suggest that the main effect of *Bauhinia megalandra* extract is on the glucose intestinal absorption without any important effect on insulin release nor on the tissue glucose consumption.

We tested the effects of: quercetin 3-*O*- α -rhamnoside (**2**), kaempferol 3-*O*- α -rhamnoside (**3**) and kaempferol 3-*O*- α -(2''-galloyl)rhamnoside (**6**), presented in Table 3, with the intention to establish the compound, present in *Bauhinia megalandra* leaves, responsible for the inhibition of the glucose intestinal absorption. The glucose intestinal absorption was measured using the method described above (Gonzalez-Mujica, *et al.*, 2003) in the absence (control) or the presence of 5 mM of the compounds **2**, **3** or **6**; phlorizin at a concentration of 0.1 mM was used as a positive control. The combined effect of kaempferol 3-*O*- α -rhamnoside (**3**) and 0.1 mM phlorizin was also studied (Rodríguez *et al.*, 2010). The kinetic parameters, K_M and V_{MAX} , of glucose intestinal absorption were measured using increasing glucose concentration (12.5 - 50 mM), in the absence and in the presence of 5 mM kaempferol 3-*O*- α -rhamnoside (**3**), but reducing the time of absorption to 15 min in order to guarantee measurement at the initial velocity. K_M and V_{MAX} were calculated from the Michaelis and Menten graph (Rodríguez *et al.*, 2010) using the Enzfitter program (Leatherbarrow, 1989).

As shown in Table 6, kaempferol 3-*O*- α -rhamnoside (**3**) exerts a statistically significant inhibition of glucose intestinal absorption, which was more than double that exhibited by quercetin 3-*O*- α -rhamnoside (**2**), inhibition also statistically significant; on the other hand kaempferol 3-*O*- α -(2''-galloyl)rhamnoside (**6**) lacked any effect.



* Means difference statistically significant at $p < 0.005$.

Fig. 12. Effects of *Bauhinia megalandra* extract on oral and subcutaneous glucose tolerance. Rats under light anesthesia (30mg of sodium pentobarbital/Kg of body weight) received 1 g/Kg body weight of glucose by gastric catheter (A) or by subcutaneous injection (B) together with 260 mg of *Bauhinia megalandra* leaf extract (-●-) or an equivalent amount of water (-■-) by gastric catheter. At the indicated times, blood samples were taken for glucose determination. The glycaemia at time zero was 75.0 ± 9.4 mg/dl. The results are expressed as the percentage of change in relation to zero-time value (100 %) and represent the means \pm standard deviation of 7 experiments with at least 2 rats in each one.

Regarding chemical structure and related biological activity, it is interesting to note that the presence of the galloyl group bound to the rhamnosyl moiety in kaempferol 3-*O*- α -(2''-galloyl)rhamnoside (6) annuls the inhibitory effect of kaempferol 3-*O*- α -rhamnoside (3). The rhamnosyl moiety is present in both kaempferol 3-*O*- α -rhamnoside (3) and quercetin 3-*O*- α -rhamnoside (2) but only the first compound efficiently inhibit glucose intestinal absorption,

indicating that the hexose is not a determinant for biological activity. The presence of an H (kaempferol 3-*O*- α -rhamnoside) in stead of an OH (quercetin 3-*O*- α -rhamnoside) in the 3' position of the flavonoid B ring is a determinant for the biological activity.

Compound	Glucose absorption nmol/30 min	% inhibition
Control	933, 4 \pm 44.3	
Quercetin 3- <i>O</i> - α -rhamnoside (2)	822.2 \pm 22.1 *	12
kaempferol 3- <i>O</i> - α -rhamnoside (3)	666.7 \pm 4.5 **	29
Kaempferol 3- <i>O</i> - α -(2''-galloyl)rhamnoside (6)	895.6 \pm 17.8 ^{NS}	4
Phlorizin	700.0 \pm 71.1 **	25
kaempferol 3- <i>O</i> - α -rhamnoside (3) + Phlorizin	300.0 \pm 44.3 ***	68

Table 6. Effects of flavonoids purified from *Bauhinia megalandra* leaves and phlorizin on the glucose intestinal absorption. Isolated intestinal segments were injected *in situ* with 1 mL of 10 mM glucose and 0.9 % NaCl for control. In the test procedures the flavonoids: quercetin 3-*O*- α -rhamnoside (2), kaempferol 3-*O*- α -rhamnoside (3) or Kaempferol 3-*O*- α -(2''-galloyl)rhamnoside (6) were added at a final concentration of 5 mM. The final concentration of phlorizin used was 0.1 mM, and the combination of 5 mM kaempferol 3-*O*- α -rhamnoside (3) and 0.1 mM phlorizin was also used. After 30 min the glucose absorbed was estimated. The results correspond to the means \pm standard deviation of 4 experiments. * Indicate $p < 0.05$, **Indicate $p < 0.005$ ***indicate $p < 0.001$ and NS not significant, according to the Student t test.

As it can be seen in Table 6, the combined use of kaempferol 3-*O*- α -rhamnoside (3) and phlorizin inhibits glucose intestinal absorption by more than double the effect of either of these alone, indicating an additive effect of both compounds. Due to the fact that phlorizin is a known inhibitor of the sodium-glucose cotransporter 1 (Panayatova-Heiermann, *et al.*, 1995) in consequence kaempferol 3-*O*- α -rhamnoside (3) should be acting on the same transporter.

	K_M in mM	V_{MAX} in $\mu\text{mol}/\text{min}$
Control	38.93 \pm 4.92 *	42.33 \pm 5.02 ^{NS}
Kaempferol 3- <i>O</i> - α -rhamnoside (3)	67.24 \pm 11.12 *	50.04 \pm 4.59 ^{NS}

Table 7. Effect of kaempferol 3-*O*- α -rhamnoside (3) on the kinetic parameters of glucose intestinal absorption. Isolated intestinal segments were injected *in situ* with 0.9 % NaCl and increasing concentration of glucose (12.5-50 mM) for control, and in the test procedure 5 mM kaempferol 3-*O*- α -rhamnoside was added. After 15 min the glucose absorbed was estimated and the K_M and V_{MAX} were calculated from the Michaelis and Menten graph using the Enzfitter program (Leatherbarrow, 1987). The results correspond to the means \pm standard deviation of 4 experiments. * Means $p < 0.005$, ^{NS} means not significant according to the Student t test.

As shown in Table 7 the control values of the kinetic parameters of glucose intestinal absorption are different to those reported by Ader, *et al.*, (2001) and Li, *et al.*, (2006), however it is important to point out that in these two cases the K_M and V_{MAX} correspond to the sodium-glucose cotransporter function and in our case the kinetic parameters correspond to the total process of the glucose intestinal absorption. The presence of 5 mM kaempferol 3-O- α -rhamnoside (**3**) increases in 1.7 times the K_M without a significant change by the V_{MAX} of the glucose intestinal absorption, these results strongly suggest that kaempferol 3-O- α -rhamnoside (**3**) behaves as a competitive inhibitor of the intestinal sodium-glucose cotransporter 1.

6. Intestinal absorption of flavonoids from *Bauhinia megalandra*

The compounds present in the plant extract, prior to develop any biological function, have to be absorbed by the intestine and pass through the blood to reach the target tissue. With the intention of obtaining information on the intestinal absorption of the compounds present in the *Bauhinia megalandra* leaves; we studied the urine of rats that drank, during a week, the plant extract instead of water. The urine was treated with acid acetone, the supernatant dried and fractionated using different solvents and column chromatography on silica gel to yield a compound that was present neither in the plant extract nor in the urine of the control rats. The structure of the compound was established using 1H and ^{13}C NMR as being an ortho-substituted benzene; however, currently we do not know the types of radicals present (results not published). Due to the fact, that the ortho-substituted benzene was present neither in the plant extract nor in the *Bauhinia megalandra* leaf extract, this must correspond to a compound, probably a flavonoid, present in the plant extract that was absorbed at the intestine level, afterwards metabolized and excreted in the urine. This ortho-substituted benzene, is different to the phenolic acid that has been reported (Tourinho, *et al.*, 2009) are eliminated by the urine of animals that ingest flavonoids. This is direct evidence of the intestinal absorption of compounds present in the *Bauhinia megalandra* leaf extract.

7. Conclusions

The aqueous extract of *Bauhinia megalandra* leaves inhibited the gluconeogenesis and glycogenolysis, stimulated by epinephrine or dibutyl AMPc, activity of liver slices, reduced the hyperglycaemia promoted by epinephrine in the intact animal and inhibited the glucose intestinal absorption in a dose dependant way by affecting the SGLT 1. From the methanol extract of the *Bauhinia megalandra* leaves, we purified and characterized 8 flavonoids for one of which, kaempferol 3-O- α -(2''galloyl)-rhamnoside (**6**), to the best of our knowledge, this was the first report of its being isolated from nature; all of the flavonoids isolated were able to inhibit the enzyme glucose-6-phosphatase, the most active ones being those with the galloyl group with IC50 of approximately 30 μ M. Quercetin 3-O- α -(2''galloyl)-rhamnoside (**5**) behaved as a competitive inhibitor of the glucose-6-phosphatase transporter of the glucose-6-phosphate system and drastically reduced the liver gluconeogenic activity. The reduction of the glycogenolysis and gluconeogenesis might be a consequence of the inhibition of the hepatic glucose-6-phosphatase by the flavonoids present in the plant extract. Kaempferol 3-O- α -rhamnoside (**3**) was a competitive inhibitor of

the glucose intestinal absorption. In the urine of the rats that drank the *Bauhinia megalandra* leaves aqueous extract we identified an ortho-substituted benzene, probably a product of the metabolism of one of the flavonoides.

We have presented direct and indirect evidences of the intestinal absorption of compounds present in the *Bauhinia megalandra* leaf extract which are responsible for the reduction in the hepatic production of glucose and of the glucose intestinal absorption, events that are important for the reduction of the supply of glucose to the blood. As a consequence the compounds present in this plant may well be useful in the treatment of diabetic patients due to their antihyperglycaemic activity.

8. Acknowledgement

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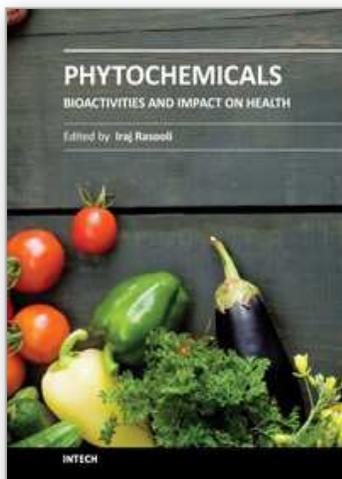
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Among the thousands of naturally occurring constituents so far identified in plants and exhibiting a long history of safe use, there are none that pose - or reasonably might be expected to pose - a significant risk to human health at current low levels of intake when used as flavoring substances. Due to their natural origin, environmental and genetic factors will influence the chemical composition of the plant essential oils. Factors such as species and subspecies, geographical location, harvest time, plant part used and method of isolation all affect chemical composition of the crude material separated from the plant. The screening of plant extracts and natural products for antioxidative and antimicrobial activity has revealed the potential of higher plants as a source of new agents, to serve the processing of natural products.

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