Chapter 4

4 Results

4.1 Crude extracts

4.1.1 Extraction of plants

Hexane extracted the lowest mass of material from the leaves of the ten *Ficus* species, while the highest mass was obtained with acetone as the extractant except for *F. cordata*, *F. natalensis* and *F. polita* for which chloroform produced the highest yield (Figure 4-1). In comparison acetone and chloroform had the same yield of extraction. The percentage yield ranged from 2.3% to 3.7% (acetone extracts), 1.6% to 3.5% (chloroform extracts), and 1.1% to 2.3% (hexane extracts). The acetone extract of *F. lutea* had the highest yield (3.7%), followed by *F. polita* (3.2%), while *F. capreifolia* had the lowest yield (2.3%). The chloroform extract of *F. polita* had the highest yield (3.5%), followed by *F. natalensis* (3.0%), while *F. capreifolia* had the lowest yield (1.6%). The hexane extract of *F. polita* had the highest yield (2.3%), followed by *F. natalensis* (2.2%), while *F. capreifolia* resulted in the lowest yield (1.1%).

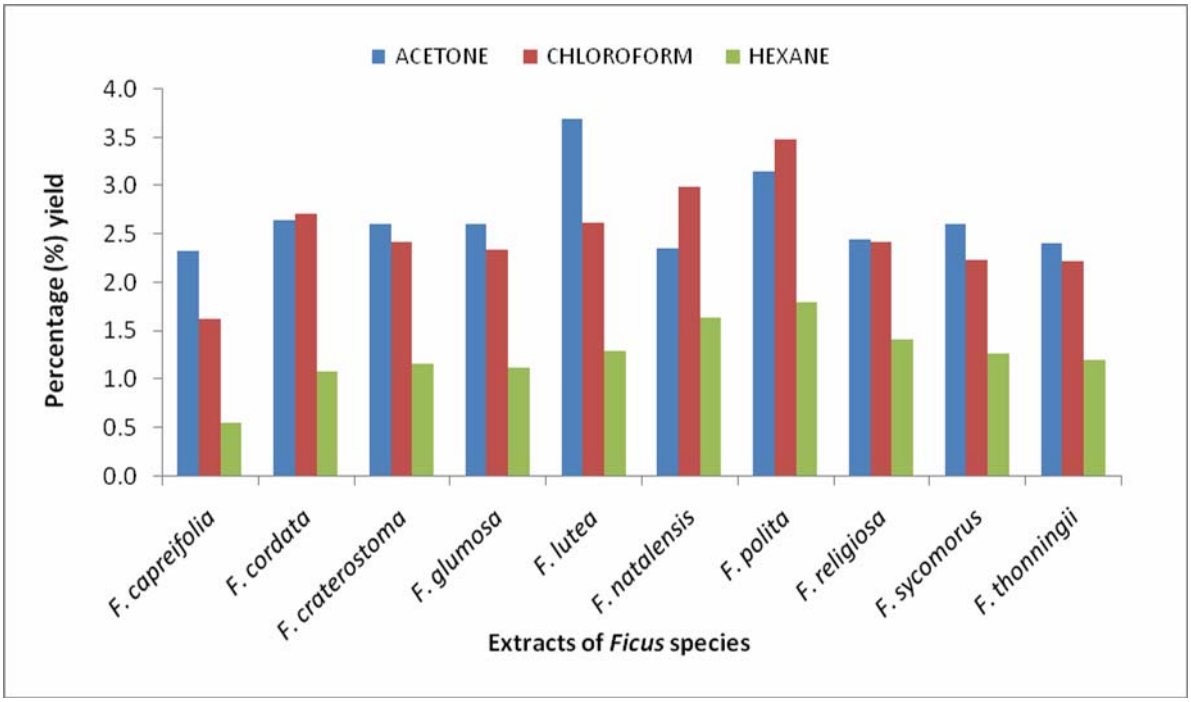


Figure 4-1Percentage extract yield (W/V) calculated as (dry extract weight/dry starting material weight) x 100. Different solvents; acetone, chloroform and hexane were used separately to extract plant material.

4.1.2 Phytochemical analysis

The TLC chromatogram of the ten *Ficus* species sprayed with vanillin-sulphuric acid produced a number of bands (Figure 4-2). The TLC chromatographic profile of the ten *Ficus* species showed many similar compounds within a solvent extractant. A comparison of the chemical profile in the form of bands for the extracts of acetone, chloroform and hexane showed that hexane had the least number of visible bands separated by the different TLC elution systems (BEA, CEF and EMW). Only the acetone extracts were developed in the FAWE eluent system as acetone is known to extract compounds which are poorly separated by the other three eluent systems used.

Acetone extracted compounds with different colours including compounds which turned red when plates were sprayed with acidified vanillin spray reagent. The BEA solvent system gave a better resolution of the compounds present in the crude extracts than CEF, EMW or FAWE. However, some compounds at the base of the plates (red in colour) which the elution systems of BEA and CEF could not resolve, probably because they are highly polar compounds, were resolved with the EMW and FAWE elution systems, with the latter being the superior system. Based on the colour reaction and the polar nature of the compounds it was suspected that these compounds were most likely polyphenolic compounds.

4.1.3 Antioxidant activity

The TLC chromatograms of the ten *Ficus* species were sprayed with 0.2% 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) in methanol as a qualitative indicator of antioxidant activity (Figure 4-3). The antioxidant TLC chromatograms of the ten *Ficus* species detected no activity in the chloroform and hexane extracts in all the elution systems (Figure 4-3). Antioxidant activity was detected in the acetone extracts with all the elution systems. For the BEA and CEF eluents, the compounds exhibiting antioxidant activity were situated at the base of the TLC plate while these compounds were better resolved with the EMW and FAWE elution systems. Antioxidant activity was detected in the acetone extracts of *F. craterostoma*, *F. glumosa*, *F.lutea*, *F. polita* and *F. sycomorus*.

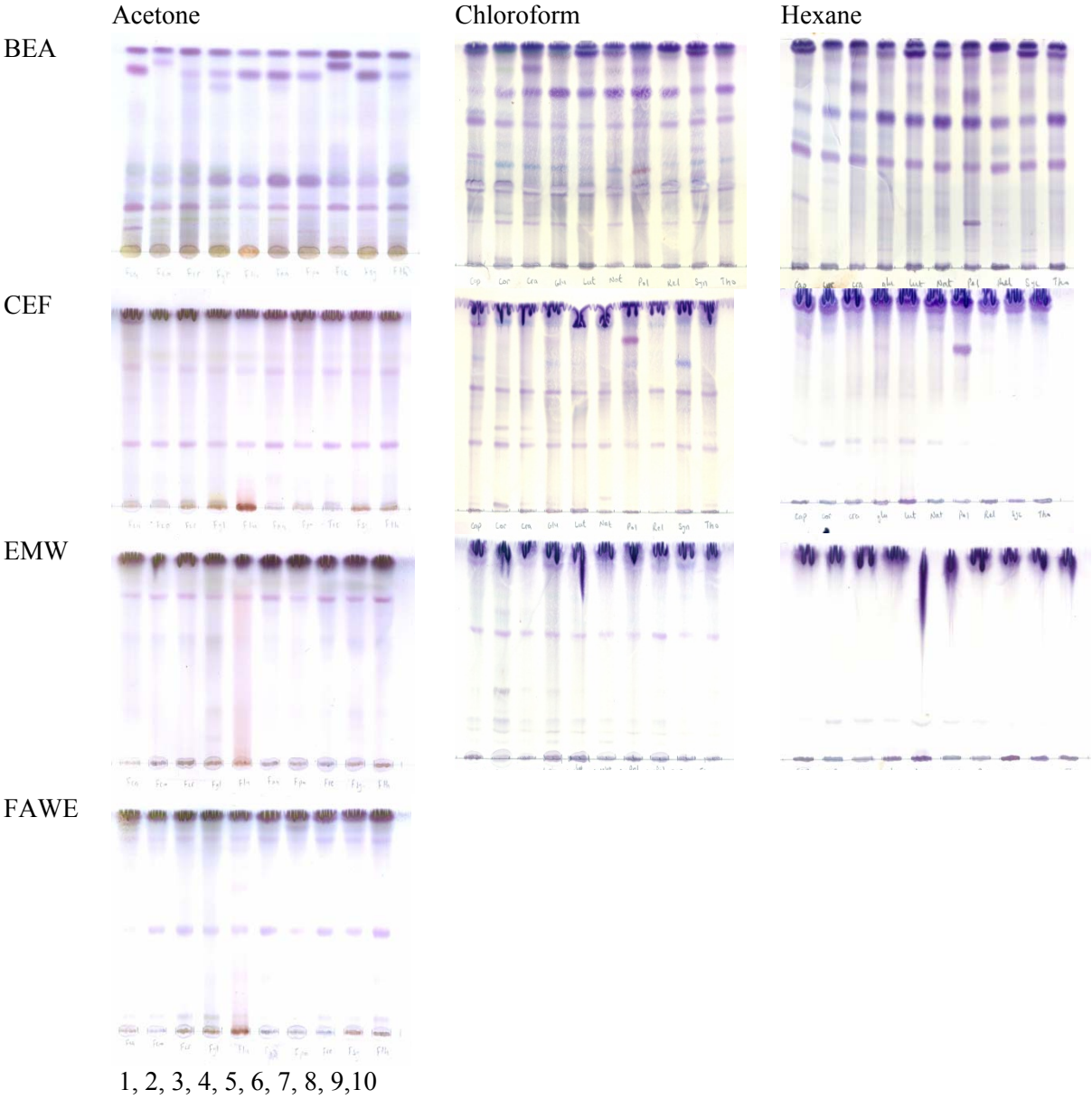


Figure 4-2TLC chromatograms of acetone, chloroform and hexane extracts of ten *Ficus* species, developed with BEA, CEF, EMW and FAWE (for acetone extracts only) sprayed with acidified vanillin to show compounds. Lanes from left to right are *F. capreifolia* (1), *F. cordata* (2), *F. craterostoma* (3), *F. glumosa* (4), *F. lutea* (5), *F. natalensis* (6), *F. polita* (7), *F. religiosa* (8), *F. sycomorus* (9), and *F. thonningii* (10).

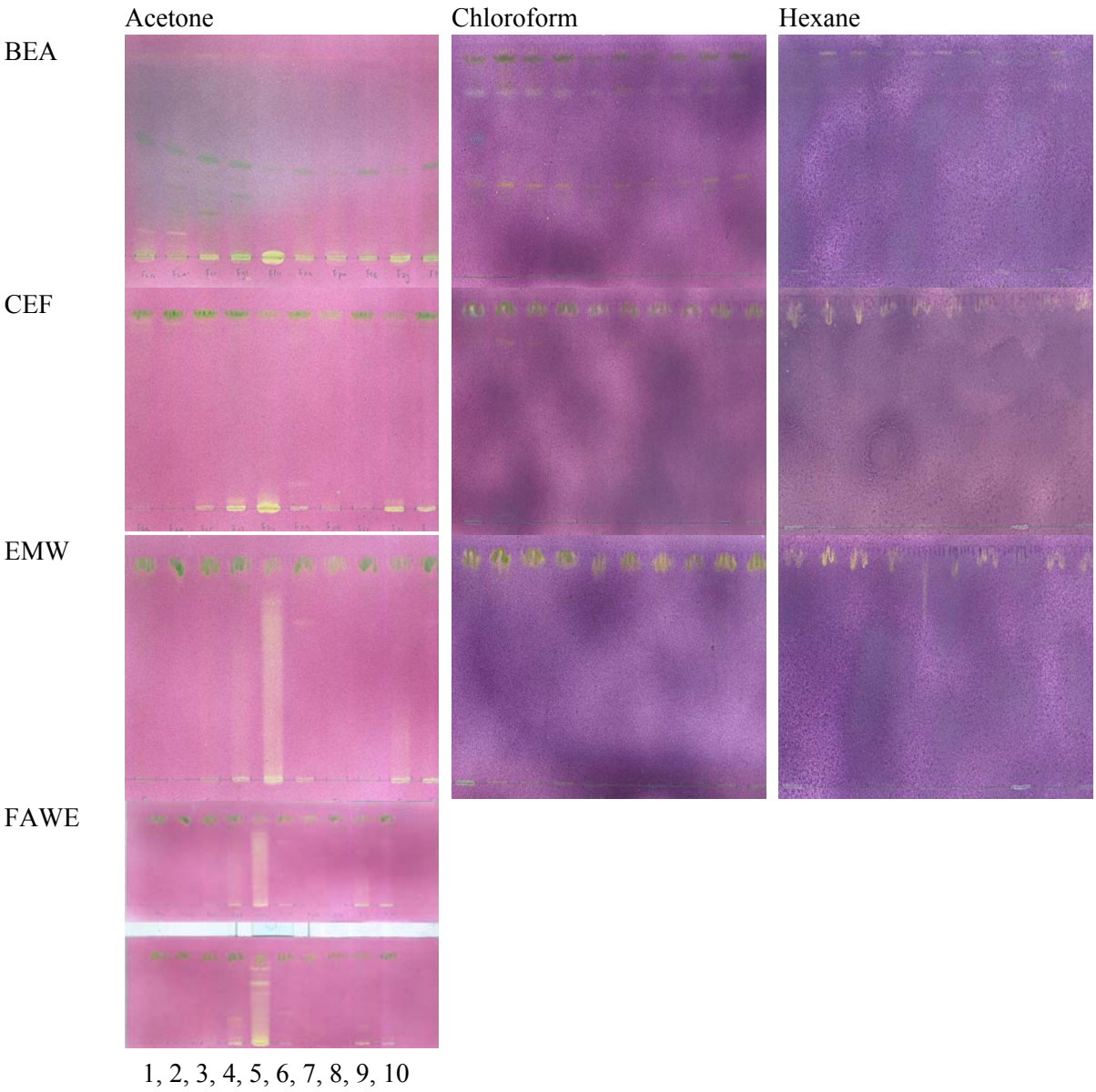


Figure 4-3Antioxidant TLC chromatograms of acetone, chloroform and hexane extracts of different *Ficus* species separated by CEF, EMW and FAWE (for acetone extracts only) sprayed with 0.2% DPPH. Clear zones indicate antioxidant activity. Lanes from left to right are *F. capreifolia* (1), *F. cordata* (2), *F. craterostoma* (3), *F. glumosa* (4), *F. lutea* (5), *F. natalensis* (6), *F. polita* (7), *F. religiosa* (8), *F. sycomorus* (9), and *F. thonningii* (10).

4.1.4 Total polyphenol content and antioxidant activity

The total polyphenol content of the crude acetone extracts of the ten *Ficus* species varied widely (Table 4-1). The total polyphenol content ranged from 4.64 to 56.85 mg GAE/g dry weight of plant. When the total polyphenol content of each extract was compared, the extract of *F. lutea* (56.85 ± 1.82 mg/g) was found to have a significantlyhigher content (p□0.001) followed in decreasing order by extracts of *F. glumosa* and *F. sycomorus* with total polyphenol content of 19.24 ± 0.79 and 12.33 ± 0.26 mg GEA/g dry weight of plant respectively. The extracts with the lowest values in decreasing

order were *F. natalensis, F. capreifolia* and *F. thonningii* with total polyphenolic content of 4.75 ± 0.92, 4.73 ± 0.26 and 4.64 ± 0.28 mg GAE/g dry weight of plant respectively.

Table 4-1Percentage yield, total polyphenol content and antioxidant activityof crude acetone extracts of leaves of ten *Ficus* species

Plants extract	[a]Total polyphenol (mg GAE/g dry weight)	[ab]Antioxidant activity TEAC
Ficus capreifolia	4.73 ± 0.26[c]	0.34 ± 0.05[c]
Ficus cordata	8.23 ± 1.00[d]	0.27 ± 0.03[c]
Ficus craterostoma	9.80 ± 0.93[d]	0.66 ± 0.06[d]
Ficus glumosa	19.24 ± 0.79[e]	1.29 ± 0.30[e]
Ficus lutea	56.85 ± 1.82[f]	4.80 ± 0.90[f]
Ficus natalensis	4.75 ± 0.92[c]	0.69 ± 0.08[d]
Ficus polita	8.04 ± 0.52[d]	0.31 ± 0.06[c]
Ficus religiosa	5.40 ± 0.35[c]	0.59 ± 0.18[c]
Ficus sycomorus	12.33 ± 0.26[e]	1.91 ± 0.19[e]
Ficus thonningii	4.64 ± 0.48[c]	0.77 ± 0.06[d]

[a]Values are means (n=9) ± standard error;
[b]Antioxidant activity (Trolox equivalent antioxidant capacity); [c,d,e,f]No significant difference between extracts with same value, but significant difference p 0.05 between different values.

The antioxidant activities of the extracts, expressed as trolox equivalent antioxidant capacity (TEAC) are presented in Table 4-1. The crude acetone extracts of the ten *Ficus* species had different antioxidant capacities (Table 4-1). The total antioxidant activity for the ten *Ficus* species varied widely from 4.80 ± 0.90 to 0.27 ± 0.03 TEAC under the assay conditions. The antioxidant activity of the extract of *F. lutea* (4.80 ± 0.90 TEAC) was significantly different (p 0.001) when compared with that of the other extracts, followed by *F. sycomorus* and *F. glumosa* in decreasing order with antioxidant activity of 1.91 ± 0.18 and 1.29 ± 0.30 TEAC respectively. The extracts with the lowest TEAC in decreasing order were *F. capreifolia*, *F. polita* and *F. cordata* with 0.34 ± 0.05, 0.31 ± 0.06 and 0.27 ± 0.03 TEAC respectively.

4.1.5 α-Amylase inhibitory activity of extracts of *Ficus* species

The crude acetone extracts of the ten *Ficus* species all demonstrated a potential to inhibit the α-amylase enzyme system (Table 4-2). The extract of *F. lutea* had the highest inhibitory potential with 95.4 ± 1.2% inhibition at concentration of 1 mg/ml. This was followed by the extract of *F. glumosa*

with65.1 ± 3.0% inhibition at the same concentration while acarbose, the positive control, had 96.7 ± 0.3% inhibition at a concentration of 0.04 mg/ml. The extracts of all the other *Ficus* species moderately inhibited α-amylase activity between 40% and 45%, except for the extracts of *F. religiosa* and *F. thonningii* with low inhibitory activities of 35.3 ± 2.8% and 37.6 ± 2.7% respectively.

Table 4-2The percentage inhibition of α-amylase activity (1 ml/mg) and concentration leading to 50% inhibition (EC_{50}) of crude acetone extracts of the ten *Ficus* species

plant	(%) α- amylase inhibition[a]	EC_{50} (µg/ml)
Ficus capreifolia	43.8 ± 3.3[b]	□100
Ficus cordata	45.9 ± 5.2[b]	□ 100
Ficus craterostoma	48.3 ± 1.6[b]	11.41 ± 4.68[b]
Ficus glumosa	65.1 ± 3.0[c]	□ 100
Ficus lutea	95.4 ± 1.2[d]	9.42 ± 2.01[b]
Ficus natalensis	43.7 ± 1.8[b]	17.85 ± 4.42[b]
Ficus polita	40.2 ± 2.6[b]	□ 100
Ficus religiosa	35.3 ± 2.8[b]	□ 100
Ficus sycomorus	40.0 ± 2.8[b]	□ 100
Ficus thonningii	37.6 ± 2.7[b]	□ 100
Acarbose	96.7 ± 0.3	0.04 ± 0.03

[a]% α-amylase inhibitory activity of crude acetone extracts of ten South African *Ficus* species (1 mg/ml) and control (acarbose) (0.04 mg/ml). Results expressed as % mean ± SEM (n=9).[b,c,d]No significant difference between extracts with same value, but significant difference p□0.05 between different values

The concentration of crude acetone extracts of the ten *Ficus* species and acarbose leading to inhibition of 50% of α-amylase activity (EC_{50}) is presented in Table 4-2. The extract of *F. lutea* was the most potent inhibitor with an EC_{50} of 9.42 ± 2.01 µg/ml followed by *F. craterostoma* (EC_{50} = 11.41 ± 4.68 µg/ml) and *F. natalensis* (EC_{50} = 17.85 ± 4.42 µg/ml) with no significant difference (p□0.05*)* among them, and acarbose with a value of 0.04 ± 0.03 µg/ml. The EC_{50} values for the extracts of the other *Ficus* species were above 100 µg/ml and therefore had limited anti α-amylase activity.

The correlation coefficient between total polyphenolic content and inhibition of α-amylase activity by the crude acetone extracts of the ten *Ficus* species R^2 was 0.81 (Figure 4-4). This indicates that the inhibition of α-amylase activity by the crude acetone extracts of the ten *Ficus* species may be due to the total polyphenolic content. Conversely, the correlation coefficient between antioxidant activity (TEAC) and inhibition of α-amylase activity by the crude acetone extracts of the ten *Ficus* species R^2

was 0.46 (Figure4-5). This indicates that the compound(s) responsible for inhibition of α-amylase activity by the crude acetone extracts of the ten *Ficus* species is most likely not be responsible for the antioxidant activity although there may be some of overlap.

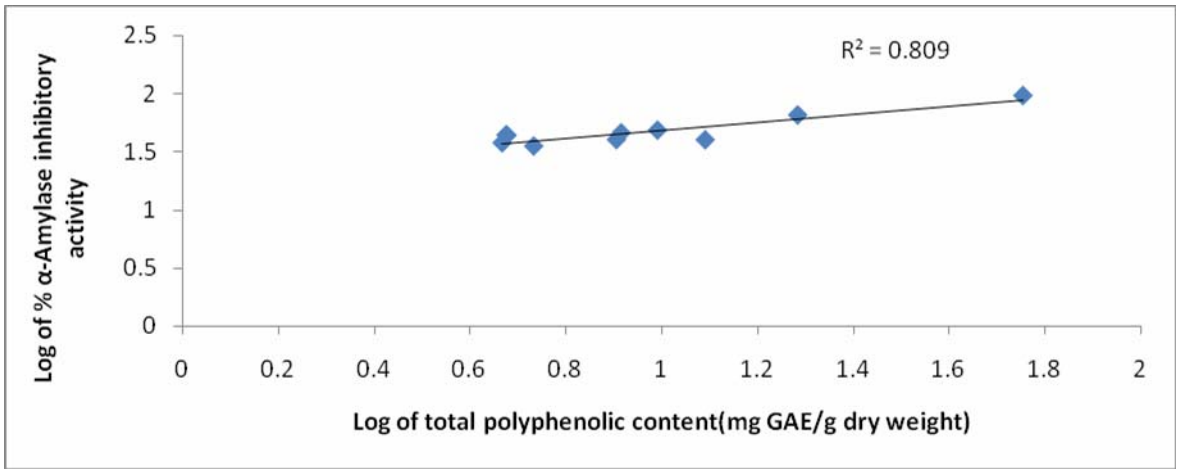


Figure 4-4The correlation between log of percentage α-amylase inhibitory activity and log of total polyphenolic contents (mg gallic acid equivalent/g dry weight of sample) of acetone extracts from the ten South African *Ficus* species.

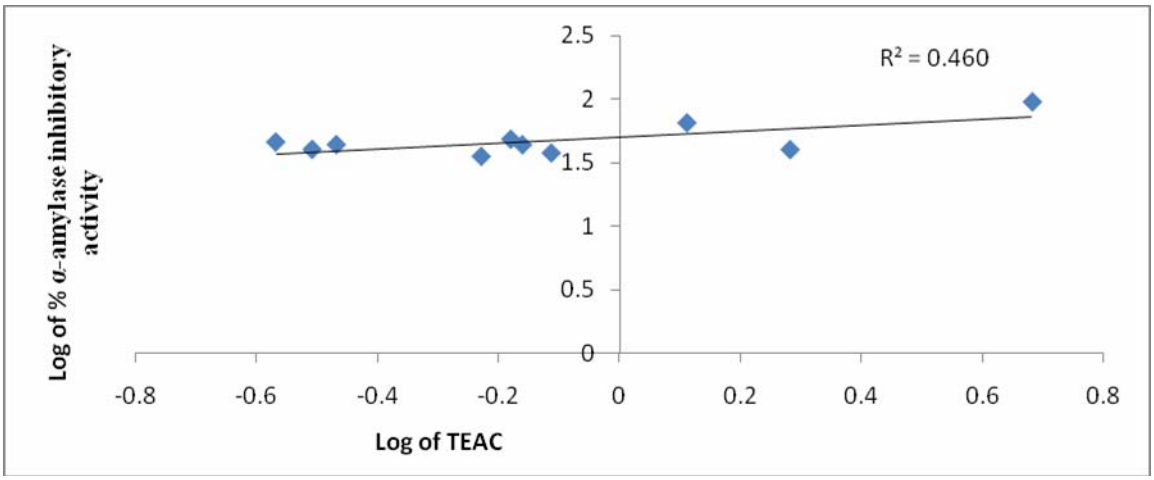


Figure4-5The correlation between log of percentage α-amylase inhibitory activity and the log of TEAC of acetone extracts from the ten South African *Ficus* species.

4.1.6 α-Glucosidase inhibitory activity of extracts of *Ficus* species

The crude acetone extracts of the ten *Ficus* species were generally weak inhibitors of α-glucosidase (Table 4-3). All the extracts of the ten *Ficus* species, except for the extract of *F. lutea*, inhibited α-glucosidasewith inhibitory activity below 40% under the assay conditions. The extract of *F. lutea* had the highest inhibitory activity of 64.3 ± 3.6% at a concentration of 0.5 mg/ml and was significantly

different (p□0.05) from the other extracts. The extracts of *F. glumosa* and *F. sycomorus* inhibited α-glucosidase activity by 38.7 ± 6.5% and 35.7 ± 5.4% respectively. The extract of *F. religiosa* inhibited α-glucosidase activitythe least (17.6 ± 8.0%). Acarbose (positive control) potently inhibited α-glucosidase activity (84.8 ± 1.7%) at a concentration of 0.02 mg/ml.

The concentration of extracts of the ten *Ficus* species and acarbose leading to inhibition of 50% of α-glucosidase activity (EC_{50}) is presented in Table 4-3. The extract of *F. sycomorus* was more potent than all the other extracts with an EC_{50} of 217 ± 69 µg/ml, followed closely by the extract of *F. lutea* (290 ± 111 µg/ml), with no significant difference between them (p□0.05). The EC_{50} of acarbose was 3.4 ± 0.5 µg/ml.

Table 4-3The percentage inhibition of α- glucosidase activity (0.5 ml/mg) and concentration leading to 50% inhibition (EC_{50}) of crude acetone extracts of ten *Ficus* species

plant	(%) α- glucosidase inhibition[a]	EC_{50} (µg/ml)
Ficus capreifolia	24.3 ± 1.7[b]	□ 1000
Ficus cordata	22.0 ± 3.6[b]	□ 1000
Ficus craterostoma	28.2 ± 7.0[b]	□ 1000
Ficus glumosa	38.7 ± 6.5[b]	□ 1000
Ficus lutea	64.3 ± 3.6[c]	290 ± 111[b]
Ficus natalensis	23.6 ± 8.1[b]	□ 1000
Ficus polita	29.2 ± 5.8[b]	□ 1000
Ficus religiosa	17.6 ± 8.0[b]	□ 1000
Ficus sycomorus	35.7 ± 5.4[b]	217 ± 69[b]
Ficus thonningii	25.3 ± 5.0[b]	□ 1000
Acarbose	84.8 ± 1.7	3.4 ± 0.5

[a]% α-glucosidase inhibitory activity of crude acetone extracts of ten South African *Ficus* species (0.5 mg/ml) and control (acarbose) (0.02 mg/ml). Results expressed as % mean ± SEM (n=9);
[b,c]No significant difference between extracts with same value, but significant difference p□0.05 between different values

The correlation coefficient between total polyphenolic content and inhibition of α-glucosidase activity by the crude acetone extracts of the ten *Ficus* species R^2 was 0.85 (Figure 4-6). This indicates that the inhibition of α-glucosidase activity by the crude acetone extracts of the ten *Ficus* species may be ascribed in large part to the total polyphenolic content.In addition, the correlation coefficient between antioxidant activity (TEAC) and inhibition of α-glucosidase activity by the crude acetone extracts of the ten *Ficus* species R^2 was 0.67 (Figure 4-7). This indicates that the compound(s) responsible for the

inhibition of α-glucosidase activity by the crude acetone extracts of the ten *Ficus* species may possible be responsible for the antioxidant activity.

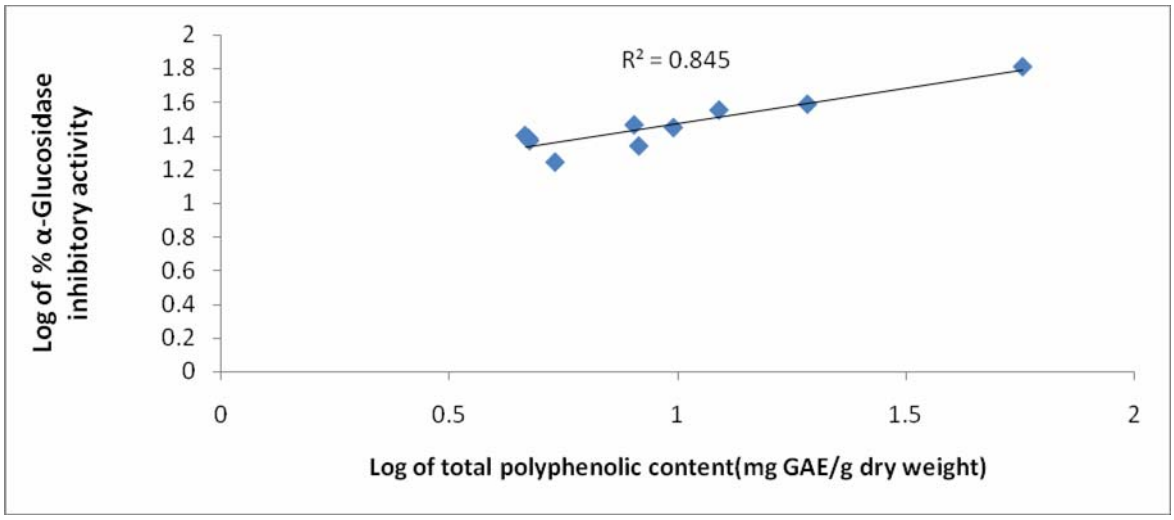


Figure 4-6The correlation between log of percentage α-glucosidase inhibitory activity and log of total polyphenolic contents (mg gallic acid equivalent/g dry weight of sample) of acetone extracts from ten South African *Ficus* species.

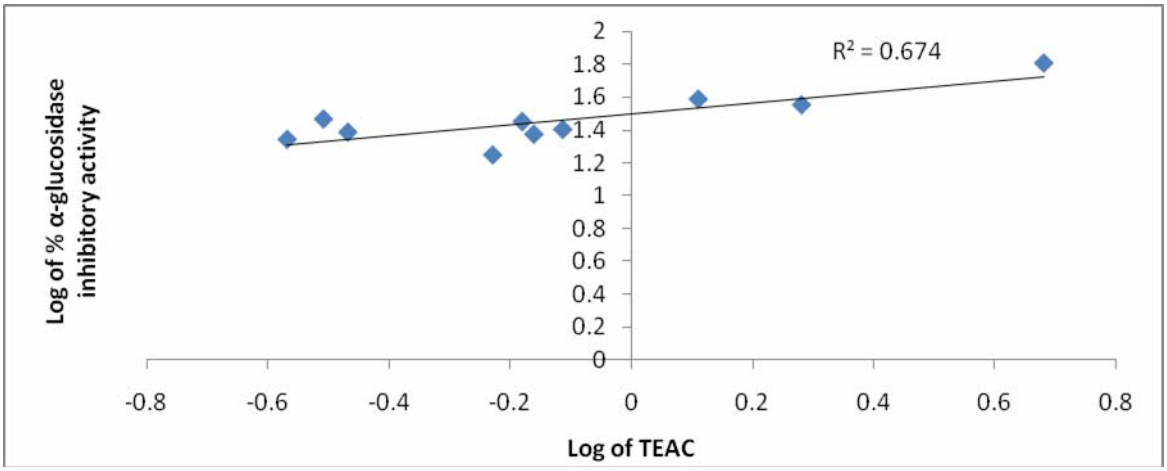


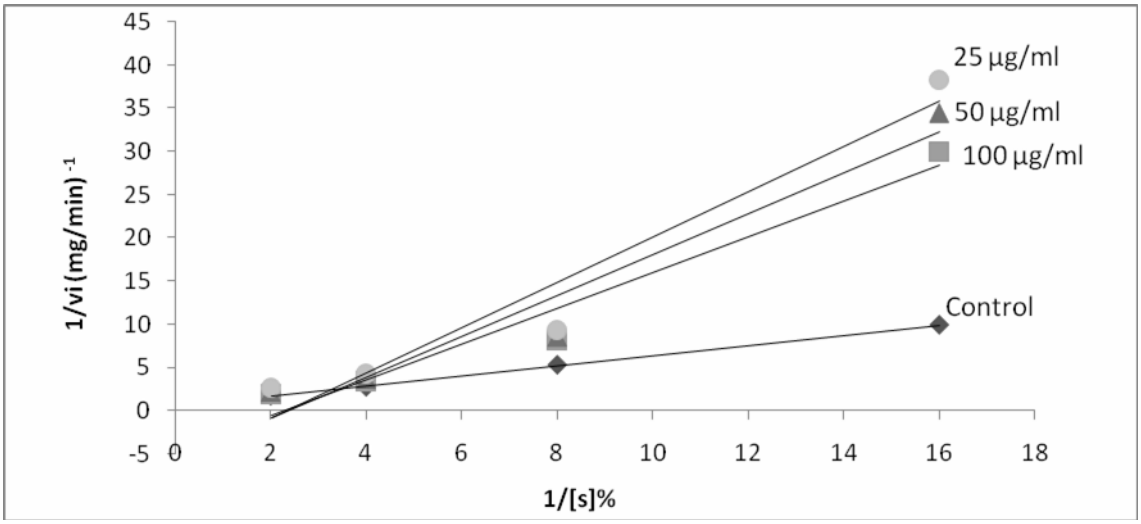
Figure 4-7The correlation between log of percentage α-glucosidase inhibitory activity and the log of TEAC of acetone extracts from the ten South African *Ficus* species.

4.1.7 The enzyme kinetics of α- amylase and α- glucosidase inhibition by extract of *F. lutea*

Analysis of the α-amylase and α-glucosidase kinetics by the crude acetone extract of *F. lutea* is shown in Figure 4-8. For the α-amylase (Figure 4-8A) and α-glucosidase (Figure 4-8B) inhibition by *F. lutea*, the intersection of the double reciprocal plot was seated at a point above the +1/[s] axis, indicating that *F. lutea* acts as a partially non-competitive-type inhibitor of α-amylase and α-glucosidase, indicating

that the active chemical(s) within the plant function was due the chemical inactivation of the enzyme, most likely via protein precipitation.

(A)



(B)

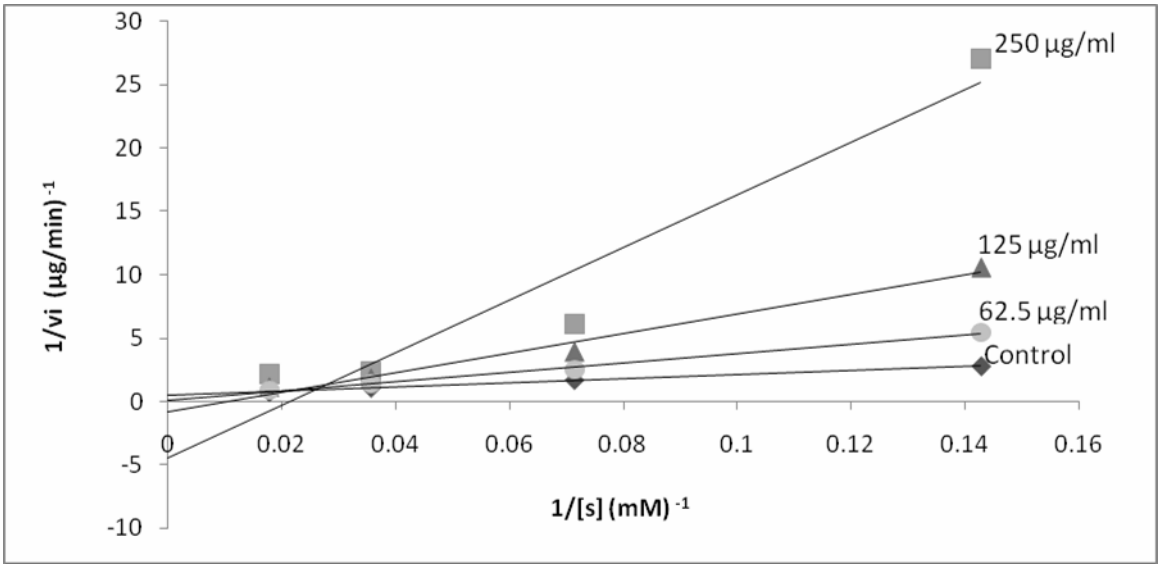


Figure 4-8Lineweaver-Burk double reciprocal plots for kinetic analysis analysis of enzyme reactions.

(A) The inhibition of porcine pancreatic α-amylase. The reciprocal of initial velocity (1/vi) of substrate (starch) versus reciprocal of different substrate concentration (%) (1/ [S]).(B) Inhibition of rat intestinal α-glucosidase.The reciprocal of initial of initial velocity (1/vi) of substrate (sucrose) versus reciprocal of different substrate concentration (mM^{-1}) (1/ [S]) in the absence and presence of different concentrations of extract of *Ficus lutea.*

4.1.8 Cytotoxicity of the acetone extracts of the ten *Ficus* species

The cytotoxic effect of the acetone extracts of the ten *Ficus* species against the Vero monkey kidney and C3A human liver cell lines are presented in Table 4-4. The extracts were less cytotoxic against the

Vero monkey kidney and C3A cell lines when compared to doxorubicin, the positive control. For the Vero kidney cells, the extract of *F. craterostoma* (356.2 ± 9.6 µg/ml), *F. lutea* (214.8 ± 5.0 µg/ml) and *F. religiosa* (110.9 ± 8.2 µg/ml) werethe least toxic based on their LC_{50}in parenthesis while the extract of *F. thonningii* (68.0 ± 1.0 µg/ml) and *F. natalensis* (69.2 ± 8.0 µg/ml) was the most toxic based on their LC_{50}in parenthesis. For the C3A liver cells, the LC_{50} for the extract of *F. craterostoma* (> 1000 µg/ml) and *F. religiosa* (922.9 ± 4.7 µg/ml) are the least toxic in decreasing order while the LC_{50} for the extract of *F. polita* (44.8 ± 1.8 µg/ml) was the most toxic. In all assays, the extract of *F. polita* was relatively toxic, while the extract of *F. craterostoma* was the least toxic. All the *Ficus* species, except extracts of *F. lutea* and *F. polita,* were more toxic to the Vero monkey kidney cell line than the C3A cell line.

Table 4-4Cytotoxicity activity of acetone extracts of the *Ficus* species (LC_{50} in µg/ml ± SE)

Plant	Vero kidney cells	C3A liver cells
F. capreifolia	85.3 ± 2.0	108.4 ± 0.8
F. cordata	76.7 ± 1.4	166.3 ± 1.9
F. craterostoma	356.2 ± 9.6	□1000
F. glumosa	72.7 ± 9.2	127.6 ± 2.6
F. lutea	214.8 ± 5.0	126.0 ± 6.8
F. natalensis	69.2 ± 8.0	113.8 ± 7.4
F. polita	90.9 ± 1.4	44.8 ± 1.8
F. religiosa	110.9 ± 8.2	922.9 ± 4.7
F. sycomorus	101.8 ± 1.1	151.6 ± 4.3
F. thonningii	68.0 ± 1.0	491.4 ± 9.9
Doxorubicin	17.0 ± 0.1	6.7 ±0.6

Values are means of triplicate determinations done three times (n=9) ± standard error.

4.1.9 Glucose uptake activity in primary rat abdominal muscle culture

The effect of the acetone extracts of the ten *Ficus* species on glucose uptake in primary rat abdominal primary muscle culture at 1 mM glucose concentration is presented in Figure 4-9. The extract of *F. lutea* significantly (p□0.001) induceda dose related glucose uptake in muscle culture with the highest glucose uptake of 10.8 ± 1.8% at a concentration of 200 µg/ml. This was followed by extracts of *F. thonningii* (8.1 ± 0.7%), *F. natalensis* (7.0 ± 7.7%) and *F. glumosa* (5.9 ± 2.7%) at the same concentration. The extracts of the other *Ficus* species did not enhanced glucose uptake in muscle cell culture in that the extracts had values lower than that of the solvent control (DMSO) (5.7 ± 1.4%). Insulin, the positive control significantly (p□0.001) enhanced glucose uptake (35.7 ± 1.0%) in muscle

culture at the highest concentration of 100 µM. Only the extract of *F. lutea* and insulin enhanced concentration dependent glucose uptake in the primary muscle cells.

4.1.10 Glucose uptake in primary rat epididymal fat cells

The effect of the acetone extracts of the ten *Ficus* species on glucose uptake in rat epididymal fat cells at 1 mM glucose concentration is presented in Figure 4-10. The extracts of *F. lutea* and *F. glumosa*significantly (p□0.001) induced a dose related glucose uptake in the primary fat cell culture with the highest glucose uptake of 32.0 ± 8.4% and 31.6 ± 5.7% respectively for the extracts of *F. lutea* and *F. glumosa*at the highest concentration of 200 µg/ml. The extracts of the other *Ficus* species did not enhance glucose uptake in the primary fat cell culture in that the extracts had values lower than that of the solvent control (DMSO) (20.6 ± 4.2%). The extract of *F. thonningii* did not enhance glucose uptake in the primary fat cell culture at all concentrations tested. Insulin, the positive control significantly (p□0.001) enhanced dose related increase in glucose uptake with the highest uptake of 82.2 ± 2.0% resulting at a concentration of 100 µM.

4.1.11 Glucose uptake activity in C2C12 muscle cells

The effect of the acetone extracts of the ten *Ficus* species at different concentrations (15 µg/ml – 500 µg/ml) on glucose uptake in C2C12 muscle cells is presented in Figure 4-11. Only the extract of *F. lutea*, in a dose related manner, significantly (p□0.001) enhanced glucose uptake in C2C12 muscle cells with the highest glucose uptake of 14.9 ± 2.3% at the highest concentration (500 µg/ml). The extract of *F. lutea* did not, however, enhance glucose uptake in C2C12 muscle cells at concentrations below 63 µg/ml. Although the extracts of the other species failed to enhance glucose uptake at 500 µg/ml in that they had values lower than DMSO, the positive control (1.8 ± 0.3%), some species did show some activity at the lower concentrations. The extract of *F. glumosa* enhanced glucose uptake of 4.5 ± 2.6% at the concentration of 250 µg/ml and the extract of *F. thonningii* enhanced glucose uptake of 3.3 ± 4.1% at the concentration of 125 µg/ml. The extract of *F. sycomorus*did not enhance glucose uptake in C2C12 muscle cells at all of concentrations tested. Insulin, the positive control significantly (p□0.001) enhanced glucose uptake in C2C12 muscle cells with the highest uptake of 19.1 ± 3.7% at the concentration of 10 µM.

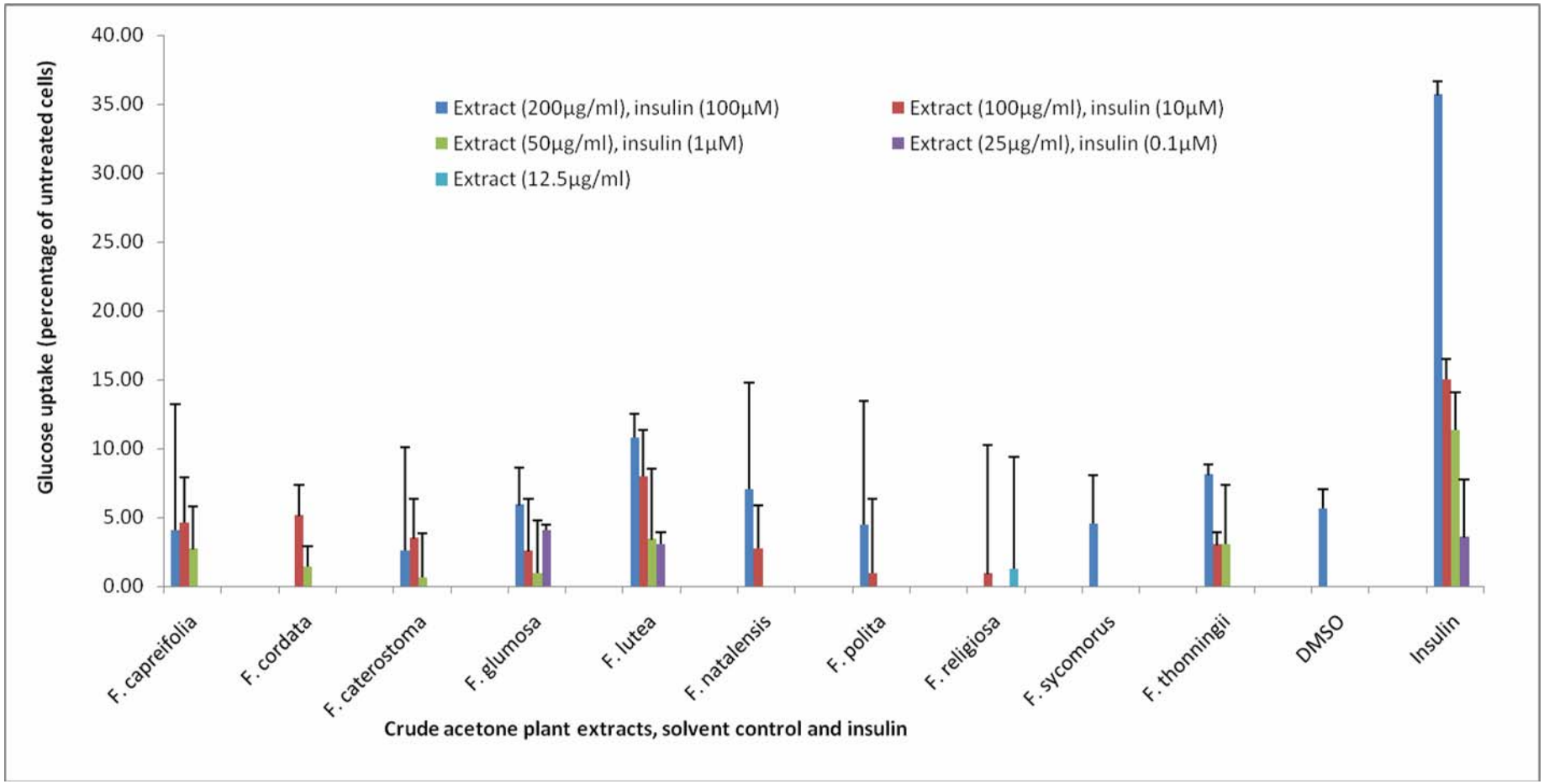


Figure 4-9Glucose uptake in rat abdominal primary muscle culture (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the acetone extracts of the ten *Ficus* species and insulin at 1 mM glucose concentration.

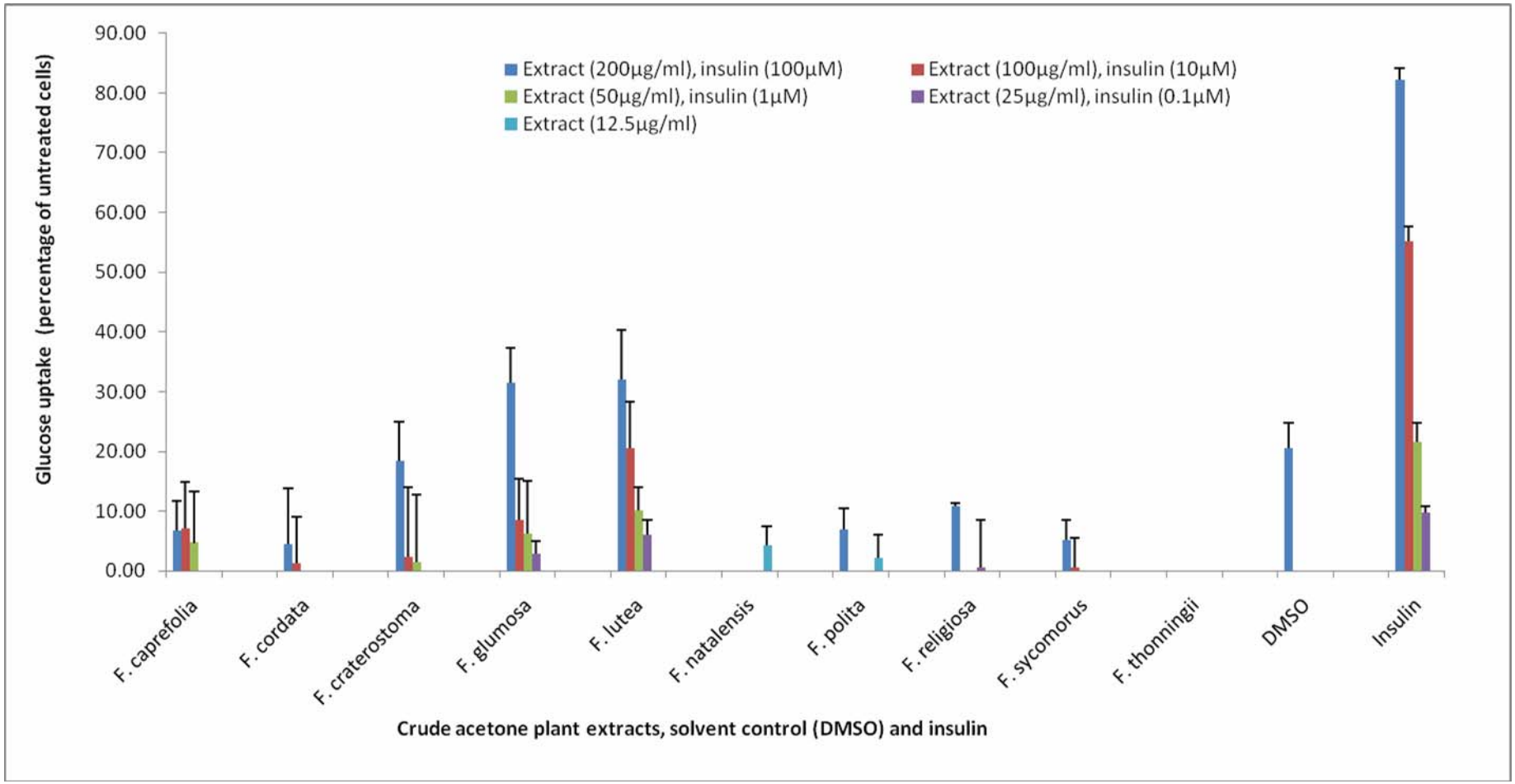


Figure 4-10Glucose uptake in rat epididymal primary fat cell culture (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the acetone extracts of the ten *Ficus* species and insulin at 1 mM glucose concentration.

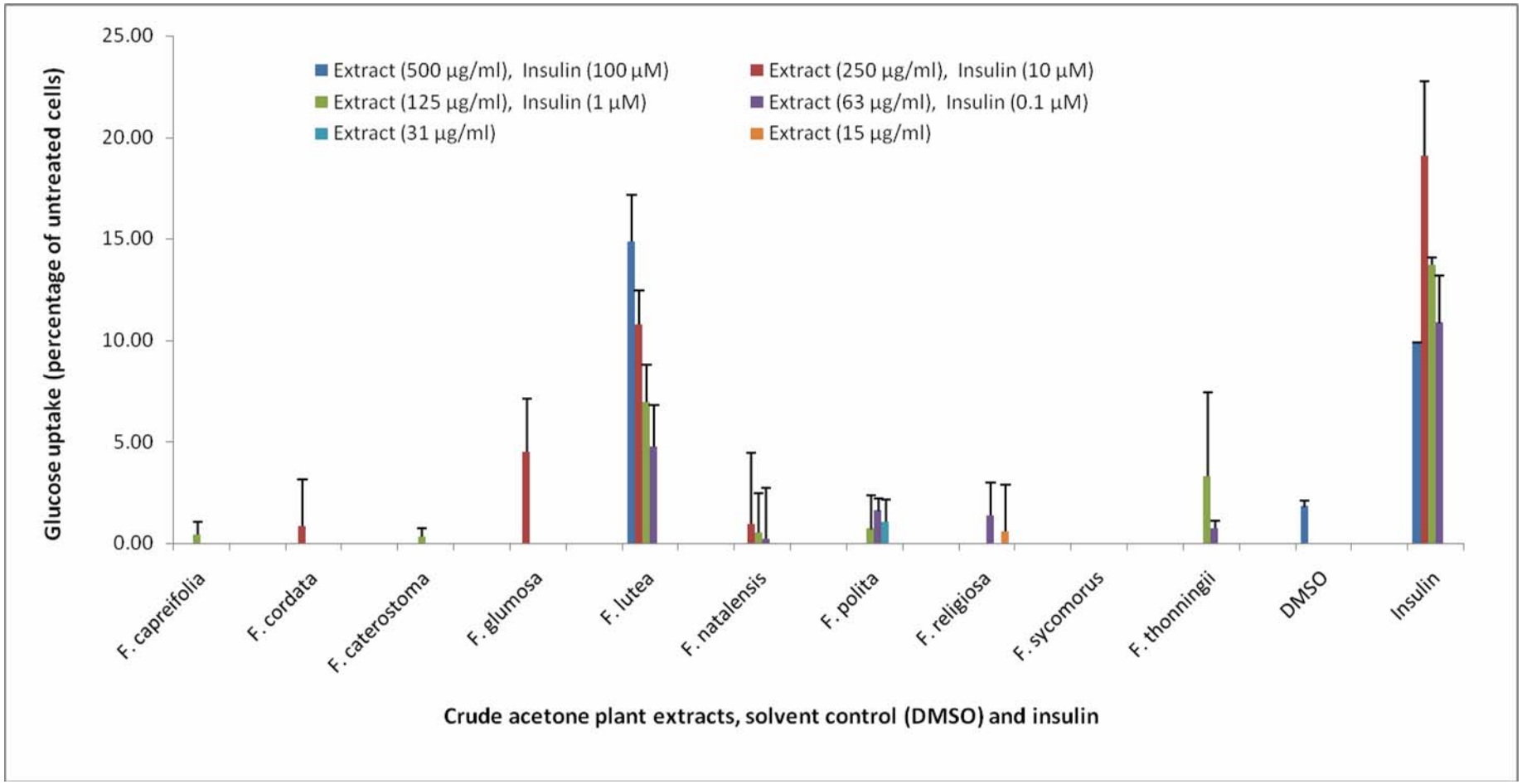


Figure 4-11Glucose uptake in C2C12 muscle cells (as percentage of untreated control cells± standard error of mean, n=9) exposed to the acetone extracts of the ten *Ficus* species and insulin.

Since the extract of *F. lutea* enhanced the highest uptake of glucose in C2C12 muscle cells, the effect of the extract on *F. lutea*(at different concentrations; 15 µg/ml – 500 µg/ml) on glucose uptake in C2C12 muscle cells in the presence of insulin at two different concentrations (1 µM and 10 µM) was investigated. The result of insulin-mediated glucose uptake in C2C12 muscle is presented in Figure 4-12. Insulin at the two concentrations tested significantly (p□0.001) influenced the glucose uptake in the C2C12 muscle cells. The medium containing the extract of *F. lutea* in the presence 1 µM and 10 µM insulin significantly (p□0.05) increased the glucose uptake in the C2C12 muscle cells (19.5 ± 0.7% and 20.8 ± 1.6% respectively) at the highest concentration of 500 µg/ml in comparison to the insulin control (14.9 ± 2.3%). For the uptake of glucose at the other concentrations of the *F. lutea* extract, the results were mixed.

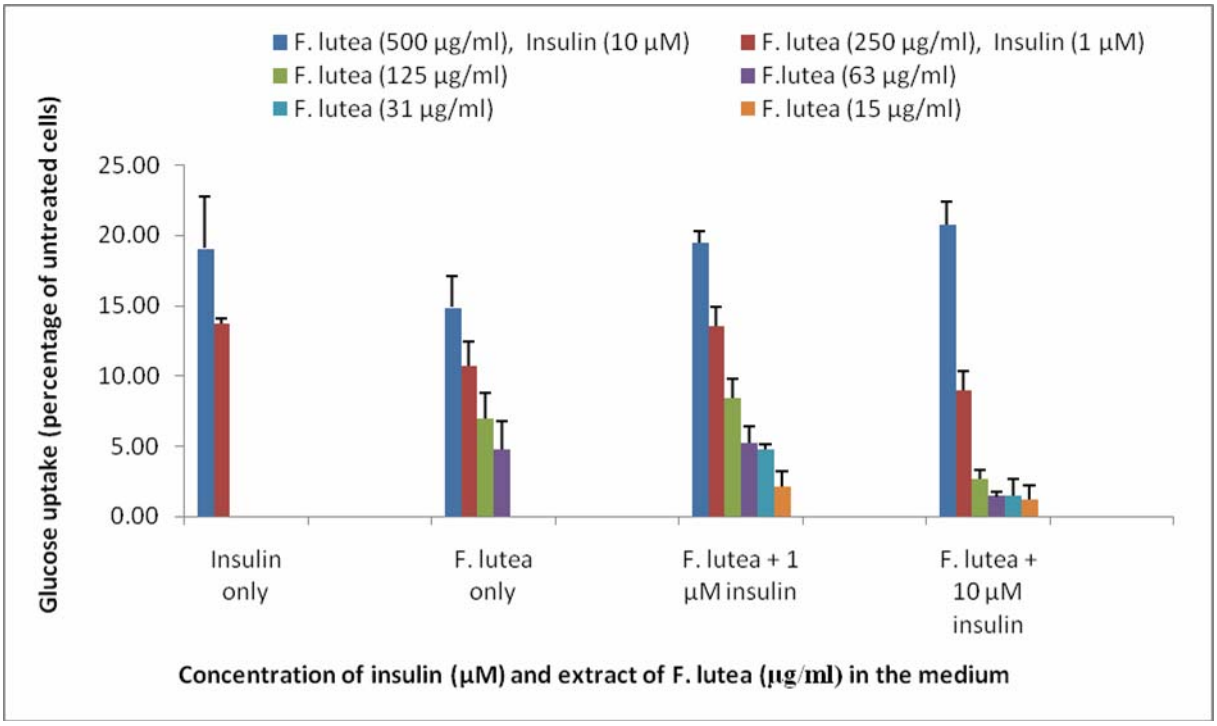


Figure 4-12Glucose uptake in C2C12 muscle cells (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the acetone extract of *F. lutea* in medium containing different concentrations of insulin (represented by the different colours) in the presence of different concentrations of insulin (x-axis).

4.1.12 Glucose uptake activity in H-4-11-E liver cells

The effect of the acetone extracts of the ten *Ficus* species at different concentrations (15 µg/ml – 500 µg/ml) on glucose uptake in H-4-II-E liver cells is presented in Figure 4-13. The extract of *F. lutea* did not significantly enhanced glucose uptake in H-4-II-E liver cells with the highest glucose uptake of glucose being only 19.3 ± 0.6% at the concentration of 500 µg/ml. None of the other concentrations enhanced glucose uptake in that their values were lower than for DMSO, the solvent control (3.4 ±

1.8%). The extracts of *F. cordata* and *F. thonningii* enhanced glucose release rather than uptake in the H-4-II-E liver cells (values below 0%) at all the concentrations tested. Metformin and insulin were used as positive controls. Metformin had a maximum glucose uptake of 18.1 ± 0.6% at the highest concentration of 100 µM while insulin significantly (p□0.001) enhanced glucose uptake in the liver cells with maximum glucose uptake of 17.7 ± 2.1% at the concentration of 10 µM.

Since the extract of *F. lutea* enhanced the highest glucose uptake in H-4-II-E liver cells, the effect of the extract on *F. lutea*(at different concentrations; 15 µg/ml – 500 µg/ml) on glucose uptake in H-4-II-E liver cells in the presence of insulin at two different concentrations (1 µM and 10 µM) was investigated. The result of insulin-mediated glucose uptake in H-4-II-E liver cells is presented inFigure 4-14. Insulin at the two concentrations tested significantly (p□0.001) influenced the glucose uptake in the H-4-II-E liver cells exposed to the extract of *F. lutea*. The medium containing insulin at 1 µM (p□0.05) and 10 µM (p□0.001) significantly enhanced glucose uptake in H-4-11-E liver cells (21.8 ± 1.6% and 24.6 ± 1.7% respectively) in the presence of the extract of *F. lutea* at 500 µg/ml in comparison to the *F. lutea* alone (19.3 ± 0.6%). The effect for the other doses was variable with no describable dose response interaction being present.The medium containing 10 µM insulin also increased glucose uptake in H-4-11-E liver cells exposed to the extract of *F. lutea* at the concentrations of 31 µg/ml and 15 µg/ml (3.2 ± 2.5% and 2.3 ± 2.3% respectively).

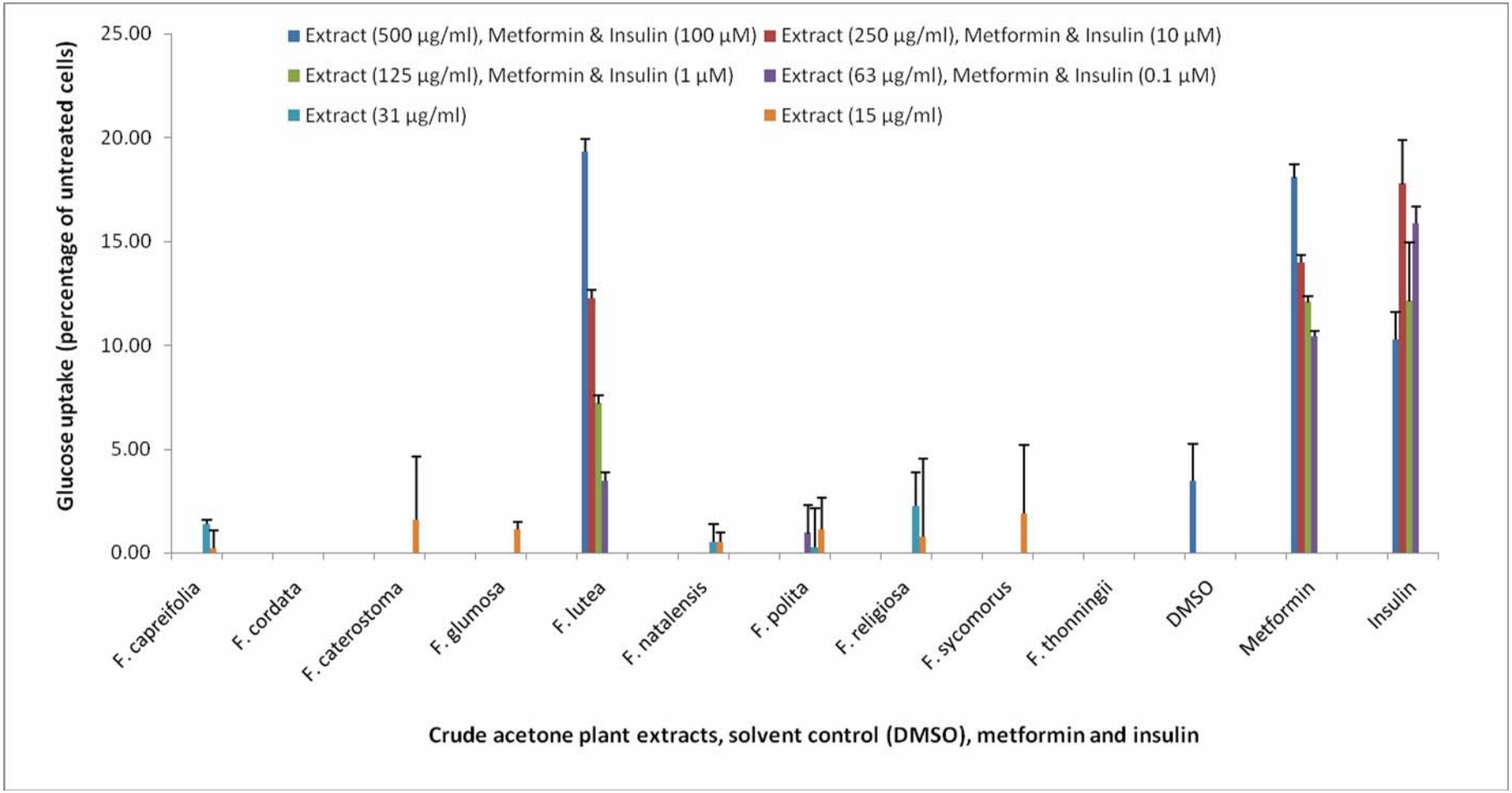


Figure 4-13Glucose uptake in H-4-11-E rat liver cells (as percentage of untreated control cells ± standard error of mean, n=9 for) exposed to the acetone extracts of the ten *Ficus* species, metformin and insulin.

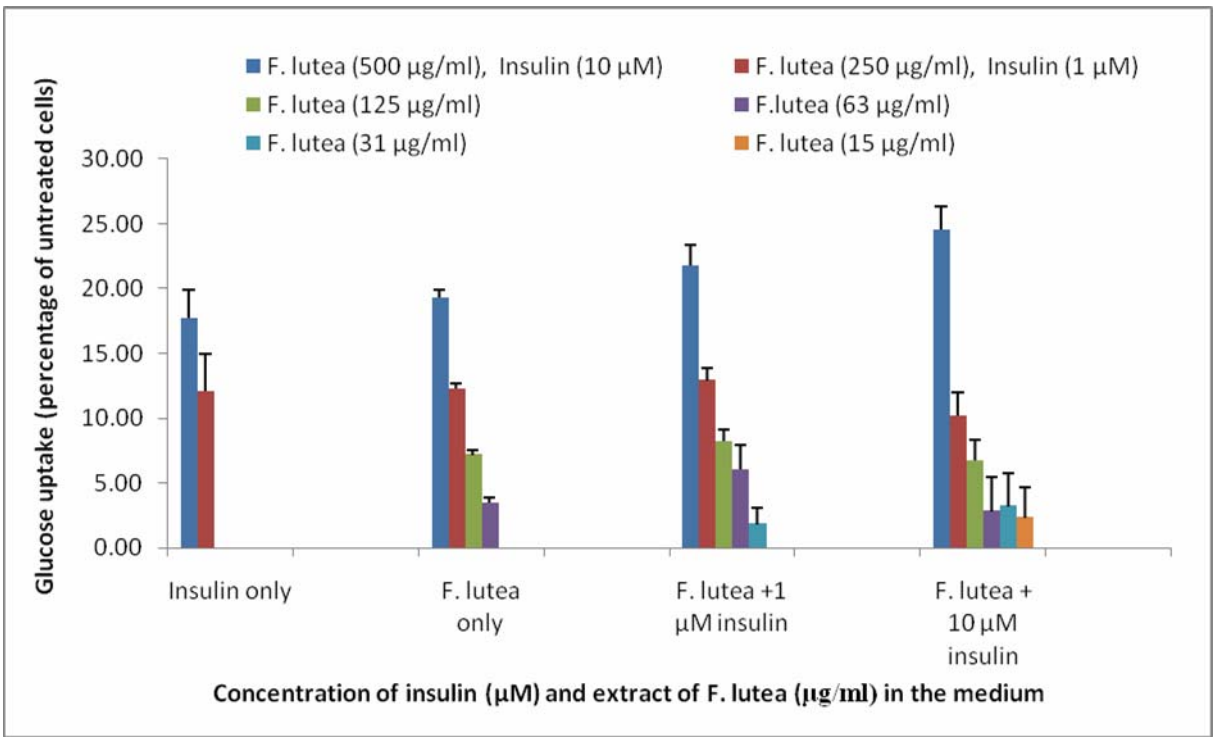


Figure 4-14Glucose uptake in H-4-11-E liver cells (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the different concentrations of the acetone extract (represented by the different colours) of *F. lutea* in medium containing different concentrations of insulin (x-axis).

4.1.13 Glucose uptake in 3T3-L1 pre-adipocytes

The effect of acetone extracts of the ten *Ficus* species at different concentrations (15 µg/ml – 500 µg/ml) on glucose uptake in 3T3-L1 pre-adipocytes is presented in Figure 4-15. None of the extracts of the *Ficus* species enhanced glucose uptake in the 3T3-L1 pre-adipocytes at the highest concentration of 500 µg/ml in that they had values lower than DMSO the solvent control.Only the extract of *F. polita* enhanced glucose uptake(7.2 ± 3.7%) in 3T3-L1 pre-adipocytes at the lowest concentration (15 µg/ml). Insulin, the positive control significantly (p□0.001) enhanced glucose uptake in 3T3-L1 pre-adipocytes with the highest glucose uptake of 23.7 ± 2.1% at the concentration 10 µM while the uptake in the DMSO treated cells were 6.5 ± 1.1%.

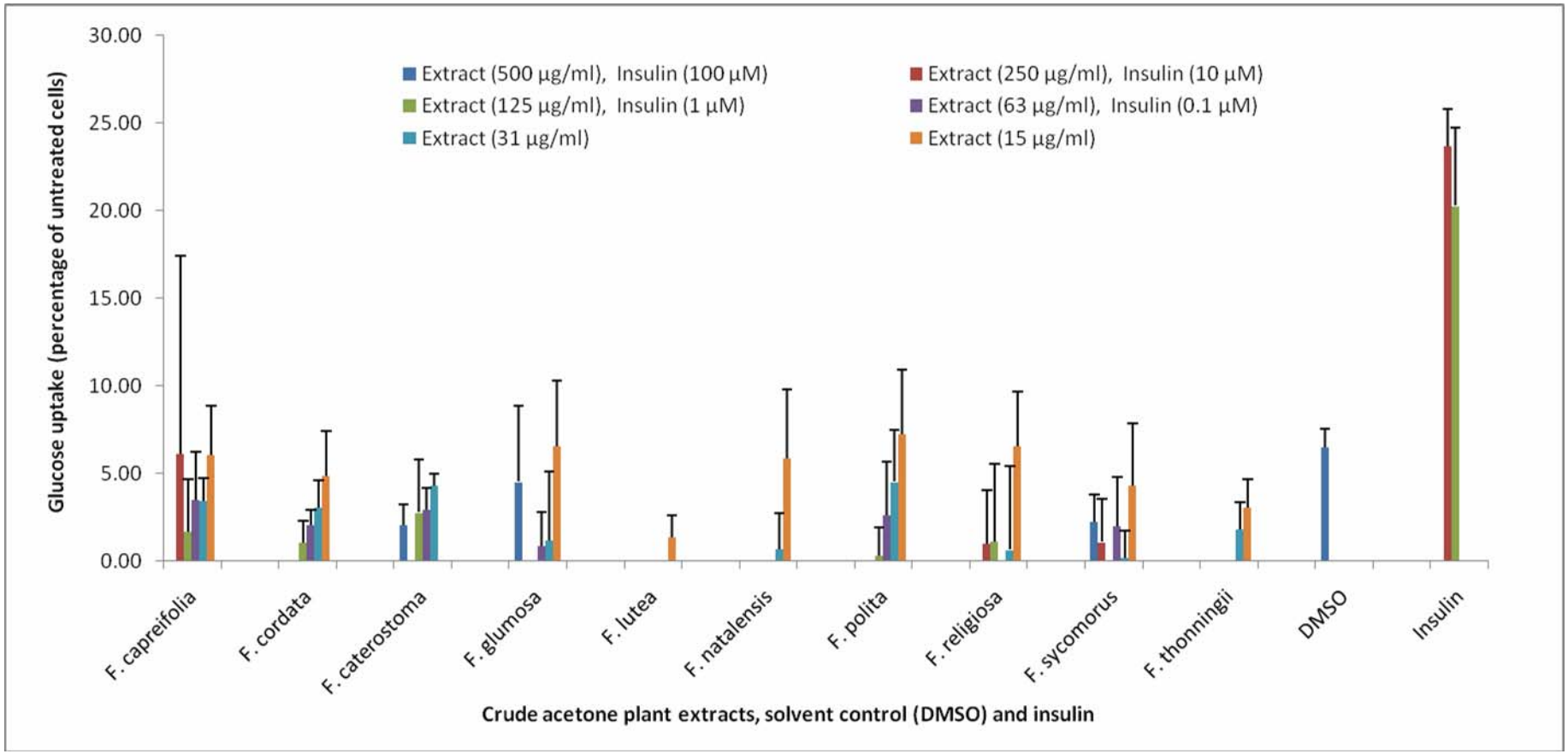


Figure 4-15Glucose uptake in 3T3-L1 pre-adipocytes (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the acetone extracts of the ten *Ficus* species and insulin.

4.1.14 Insulin secretion in RIN-m5F pancreatic cells

Since the cells (muscle and liver cells) exposed to the extract of *F. lutea* utilised more glucose than cells exposed to the extracts of the nine other *Ficus* species, the effect of extract of *F. lutea* at different concentrations (62.5 µg/ml – 500 µg/ml) on insulin secretion in RIN-m5F pancreatic β-cells incubated in medium free of glucose (non-stimulatory condition) was evaluated and was compared with the untreated control cells. The RIN-m5F pancreatic β-cells exposed to the extract of *F. lutea* resulted in a dose related increase in insulin secretion (Figure 4-16). The insulin secreted significantly (p□0.001) increased from 26.4 ± 69.7% to 120.8 ± 11.1% as the concentration of the extract of *F. lutea* was increased from 62.5 µg/ml to 500 µg/ml. Likewise the RIN-m5F pancreatic β-cells exposed to glibenclamide (positive control) significantly (p□0.001) secreted insulin in a dose dependent manner with the highest insulin secretion (142.7 ± 13.9%) at the concentration of 100 µM.

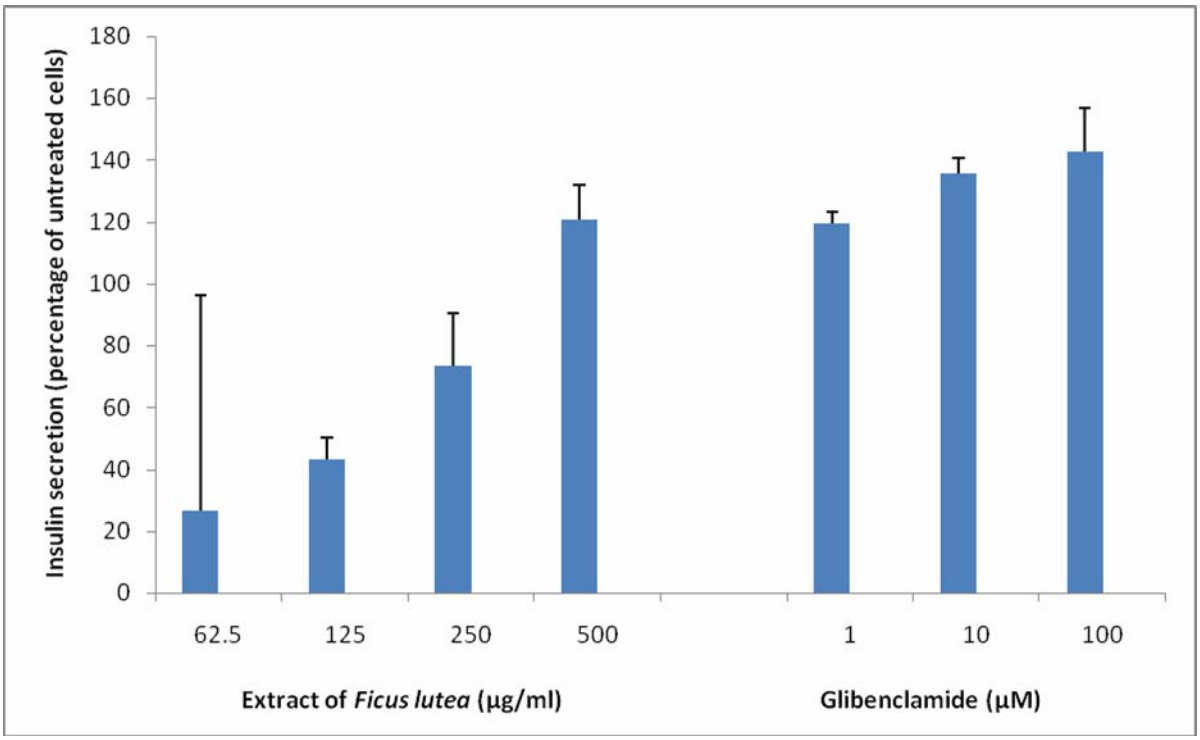


Figure 4-16Insulin secreted in RIN-m5F pancreatic cell (as percentage of untreated control cells ± standard error of mean, n=6) exposed to the acetone extract of *F. lutea* and glibenclamide (positive control) in glucose free medium.

The effect of extract of *F. lutea* on the viability of RIN-m5F pancreatic β-cells was investigatedsubsequent to the insulin secretion assay. The result showed that RIN-m5F cells exposed to the extract of *F. lutea* had a mild dose related decrease in cell viability from 98.8 ± 9.9% to 81.0 ± 1.3% as concentration was increasedfrom 62.5 µg/ml to 500 µg/ml (Figure 4-17). The correlation coefficient between the viability of RIN-m5F pancreatic β-cells and insulin secretion by the extract of *F. lutea* $R^2$was 0.72 (Figure 4-18). This indicates that the stimulation of RIN-m5F pancreatic β-cells

by the extract of *F. lutea* to secrete insulin may in part be attributed to the disruption of cell membrane.

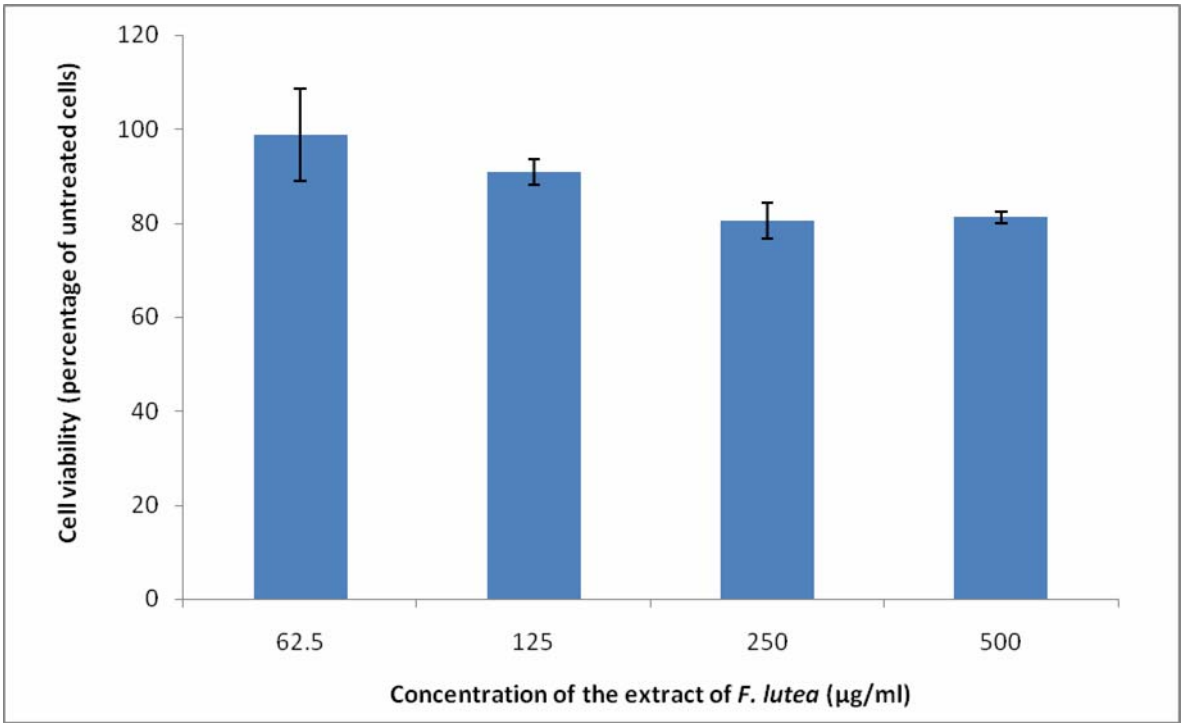


Figure 4-17Effect of the extract of *F. lutea* on RIN-m5F pancreatic cell viability (as percentage of untreated control cells ± standard error of mean, n=6) after stimulating insulin secretion

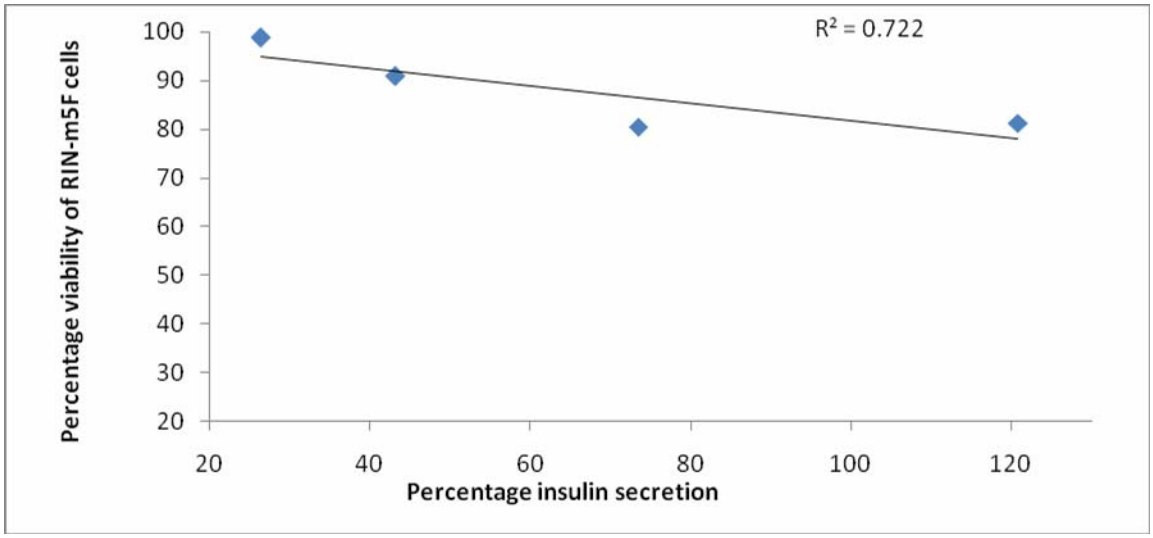


Figure 4-18The correlation between percentage cell viabilty of RIN-m5F pancreatic β-cells and percentage insulin secretion by the acetone extract of *F. lutea*.

4.2 Solvent-solvent fractionation of extract of *F. lutea*

4.2.1 Percentage yield of fractions

The powdered leaf of *F. lutea* (3g) each was weighed into two beakersand was extracted with acetone to produce about 112.5 mg of dried crude extract which was subjected to solvent-solvent fractionation. The percentage yield of the solvent-solvent fractionation of the crude acetone extract of *F. lutea* is presented (Figure 4-19). The percentage yield of ranged from 13.7% - 42.4% (15.4 mg – 47.7 mg) with hexane extracting the lowest mass 15.4 mg (13.7%) and ethyl acetate extracting the highest mass 47.7 mg (42.4%) followed by n-butanol 41.0 mg (36.4%).

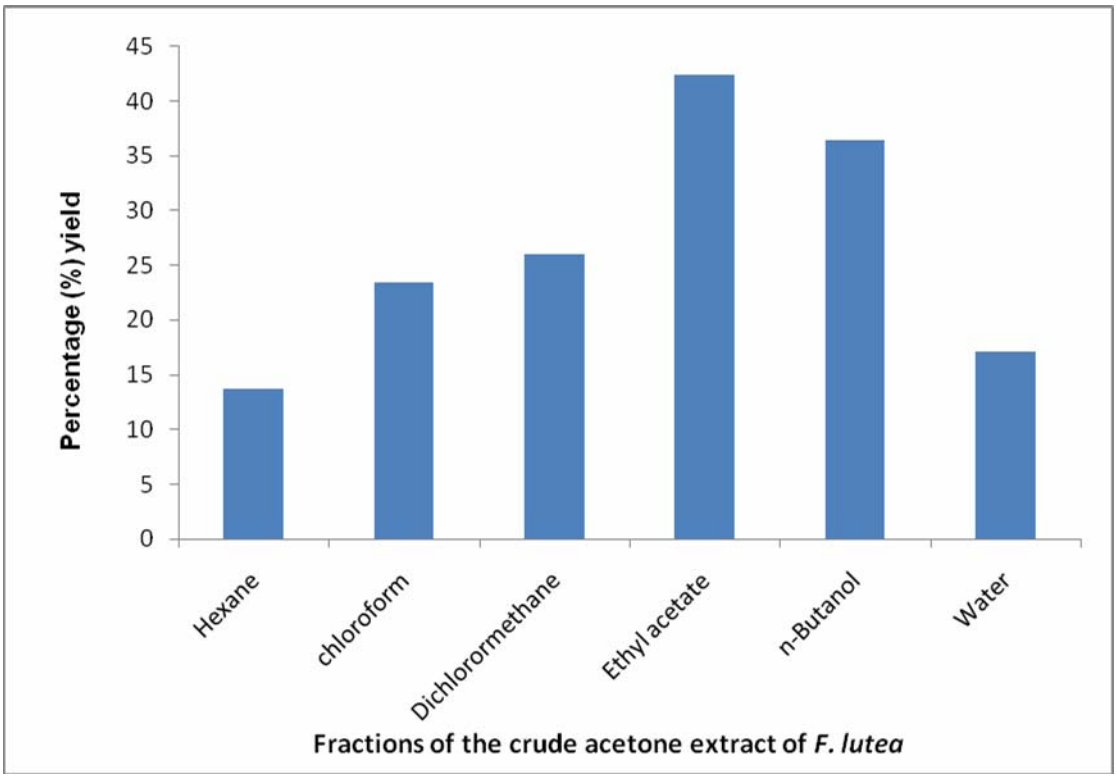


Figure 4-19Percentage yield of fractions was calculated as (dry fraction weight/dry extract weight) × 100.

4.2.2 Antioxidant activity

The TLC chromatograms of the six fractions from the acetone extract of *F. lutea* sprayed with vanillin and 0.2% 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) are presented in Figure 4-20B. For BEA and CEF elution systems, the compounds exhibiting antioxidant activity are seated at the base of the TLC plate while those compounds are better resolved with the EMW and FAWE elution system. The antioxidant TLC chromatograms of fractions did not detect significant antioxidant activity in the hexane,

chloroform and dichloromethane fractions. However, antioxidant activity was detected in the ethyl acetate, n-butanol and water fractions.

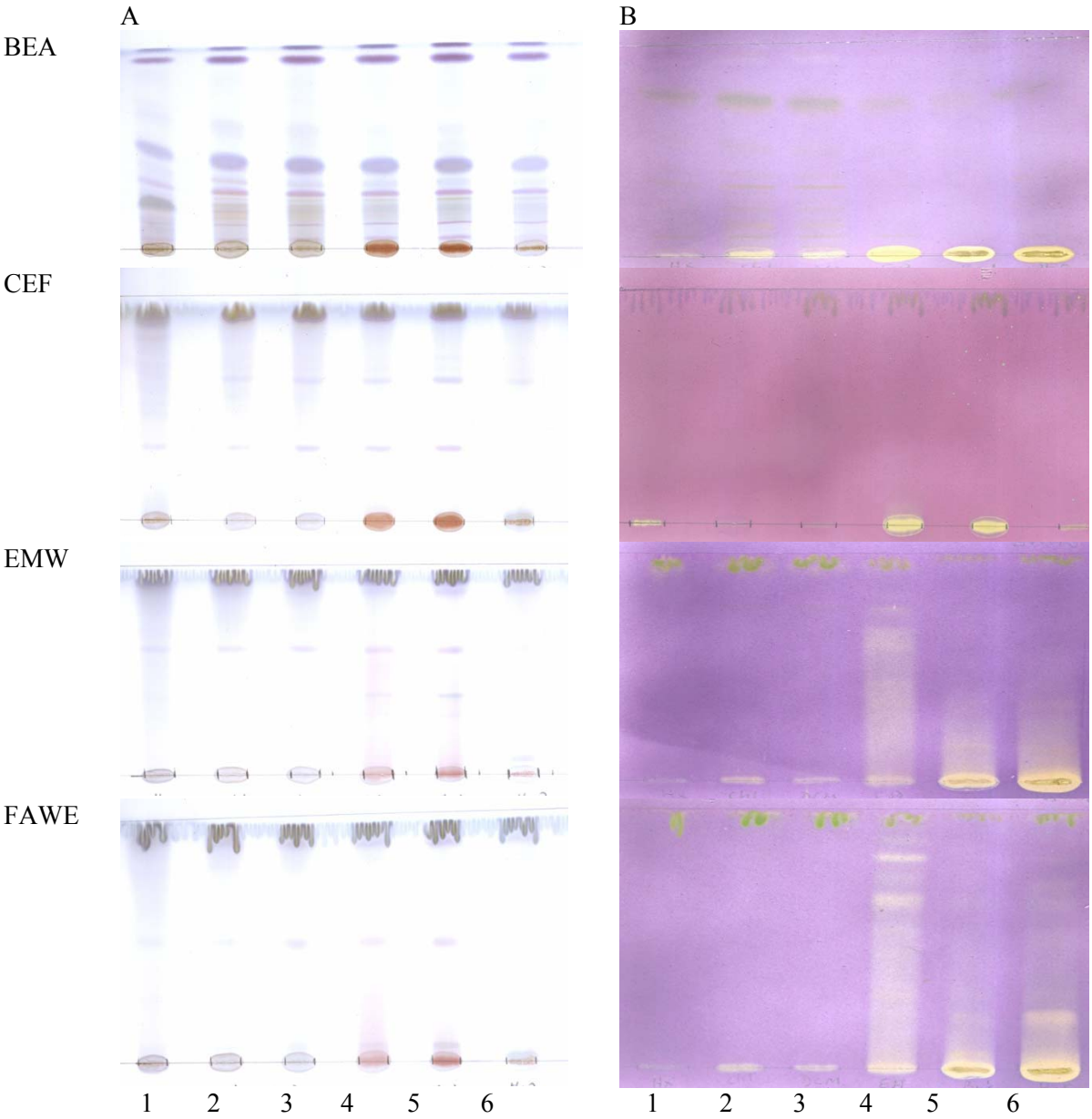


Figure 4-20TLC chromatograms of fractions from the crude acetone extract of *F. lutea* developed with BEA, CEF, EMW and FAWE sprayed with (A) acidified vanillin to show compounds and (B) 0.2% DPPH with clear zone indicating antioxidant activity. Lanes from left to right are fractions of hexane (1), chloroform (2), dichloromethane (3), ethyl acetate (4), n-butanol (5) and water (6).

4.2.3 Total polyphenol content

The total polyphenol content of the six fractions from the acetone extract of *F. lutea* varied widely (Table 4-5), ranging from 10.32 ± 0.82 to 100.51 ± 1.60 mg GAE/g dry weight of extract. When the total polyphenol content of each fractions was compared, the ethyl acetate fraction (100.51 ± 1.60

mg/g dry weight of extract) was found to have significantly higher content ($p \leq 0.001$) followed by n-butanol fraction (79.58 ± 0.50 mg/g dry weight of extract).

Table 4-5The total polyphenol contentof fractions of the crude acetone extract of *F. lutea*

Fractions	[ab]Total polyphenol (mg GEA/g dry weight extract)
Hexane	14.86 ± 1.43[c]
Chloroform	10.32 ± 0.82[c]
Dichloromethane	11.83 ± 2.32[c]
Ethyl acetate	100.51 ± 1.60[d]
n-butanol	79.58 ± 0.50[e]
water	13.34 ± 0.85[c]

[a]Values are means of triplicate determinations done three times (n=9) ± standard error;
[b]Total polyphenolic contents (mg gallic equivalent/g dry weight of extract) of crude acetone extract of *F. lutea.*
[c,d,e]No significant difference between extracts with same value, but significant difference $p \leq 0.05$ between different values

4.2.4 Inhibition of α-amylase activity by the fractions from the acetone extract of *F. lutea*

The fractions of the crude acetone extract of *F. lutea* had potential to inhibit α-amylase activity (Figure-4-21). The n-butanol fraction had the highest inhibitory potential (88.3 ± 0.8%), followed by the ethyl acetate fraction with 78.3 ± 2.5% inhibition, both at 0.5 mg/ml. The dichloromethane and water fractions inhibited α-amylase with 52.3 ± 0.5% and 34.4 ± 4.5% inhibition respectively, while hexane and chloroform inhibited α-amylase activity at between 40% and 48%.

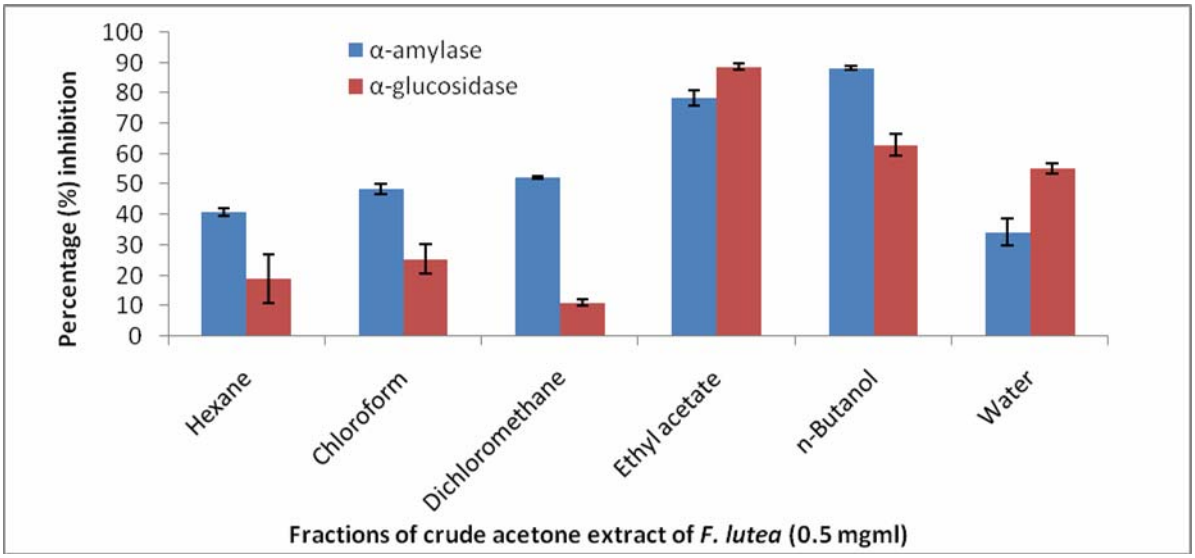


Figure 4-21The percentage inhibition of α-amylase and α-glucosidase (sucrase) activity by the six fractions of the crude acetone extract of *F. lutea* (0.5 mg/ml). Results are expressed as mean ± SEM (n=9).

The concentration of the six fractions of the extract of *F. lutea* leading to inhibition of 50% of α-amylase activity (EC_{50}) is presented in Table 4-6. The n-butanol fraction was the most potent inhibitor with an EC_{50} value of 26.50 ± 1.22 µg/ml followed by the ethyl acetate fraction (39.53 ± 7.10 µg/ml) with a significant difference between them (p□0.05*)*.

Table 4-6The EC_{50} of α-amylase and α-glucosidase activity of the fractions of the acetone extract of *F. lutea*

Fractions	α-Amylase inhibition (EC_{50}) µg/ml	α-Glucosidase inhibition (EC_{50}) µg/ml
Hexane	□1000	□1000
Chloroform	□1000	□1000
Dichloromethane	□1000	854.51 ± 56.92[a]
Ethyl acetate	39.53 ± 7.10[a]	126.78 ± 30.62[b]
n-Butanol	26.50 ± 1.22[b]	195.17 ± 63.60[b]
Water	□1000	558.40 ± 51.67[a]

[a,b,c]No significant difference between extracts with same value, but significant difference p□0.05 between different values

The correlation coefficient between total polyphenolic content and the percentage inhibition of α-amylase activity by the fractions of the acetone extract of *F. lutea* R^2 was 0.81 (Figure 4-22). Most of the points of the graph are at the lower concentration as the four fractions of *F. lutea* contained low amount of polyphenolic compounds and weak α-amylase activity inhibitor while the two fractions

contained higher amount of polyphenolic compounds and are potent inhibitors of activity of α-amylase. This indicates that the inhibition of α-amylase activity by the fractions of extract of *F. lutea* may be due to thepolyphenolic compounds therein.

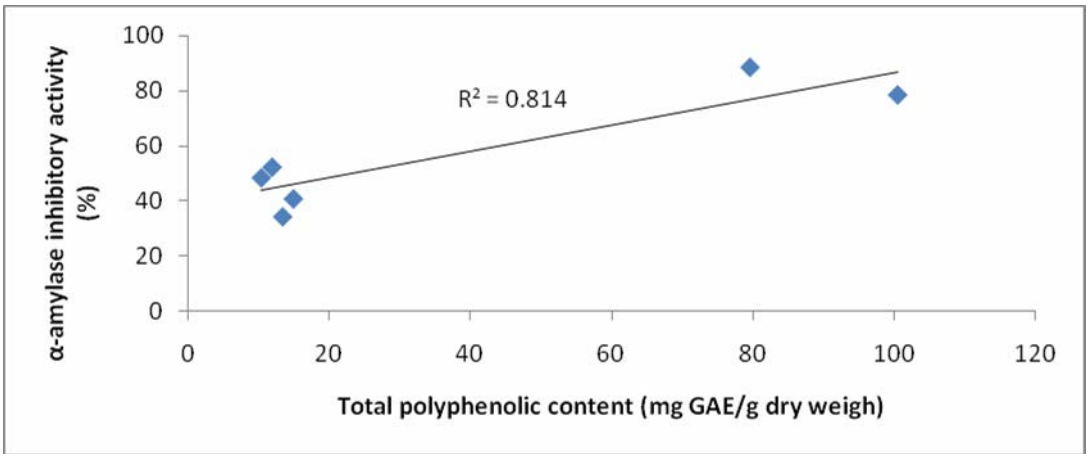


Figure 4-22The correlation between α-amylase inhibitory activities (%) and total polyphenolic contents (mg gallic acid equivalent/g dry weight of sample) of the fractions of the acetone extract of *F. lutea.*

4.2.5 Inhibition of α-glucosidase activity by the fractions of acetone extract of *F. lutea*

The fractions of the crude acetone extract of *F. lutea* inhibited α-glucosidase (sucrase) activity (Figure 4-21). The ethyl acetate fraction had the highest inhibitory potential (88.6 ± 1.0%) followed by the n-butanolic and water fractions with 63.0 ± 3.7% and 55.3 ± 1.7% inhibition respectively all at the concentration of 0.5 mg/ml. The other three fractions weakly inhibited the activity of α-glucosidase with percentage inhibition less than 30%.

The concentration of the six fractions of the extract of *F. lutea* leading to inhibition of 50% of α-glucosidase activity (EC_{50}) is presented in Table 4-6. The ethyl acetate fraction was the most potent inhibitor with an EC_{50} value of 126.78 ± 30.62 µg/ml followed by the n-butanol fraction (195.17 ± 63.60 µg/ml) with no significant difference between them (p□0.05*)*. The correlation coefficient between total polyphenolic content and the percentage inhibition of α-glucosidase activity by the fractions of the acetone extract of *F. lutea* R^2 was 0.74 (Figure 4-23). As above, most of the points of the graph are at the lower concentration as four fractions of *F. lutea* contained low amount of polyphenolic compounds and weak α-glucosidase activity inhibitor while the other two fractions contained higher amount of polyphenolic compounds and are potent inhibitors of activity of α-glucosidase. This indicates that the inhibition of α-glucosidase activity by the fractions of extract of *F. lutea* may be due to the total polyphenolic content.

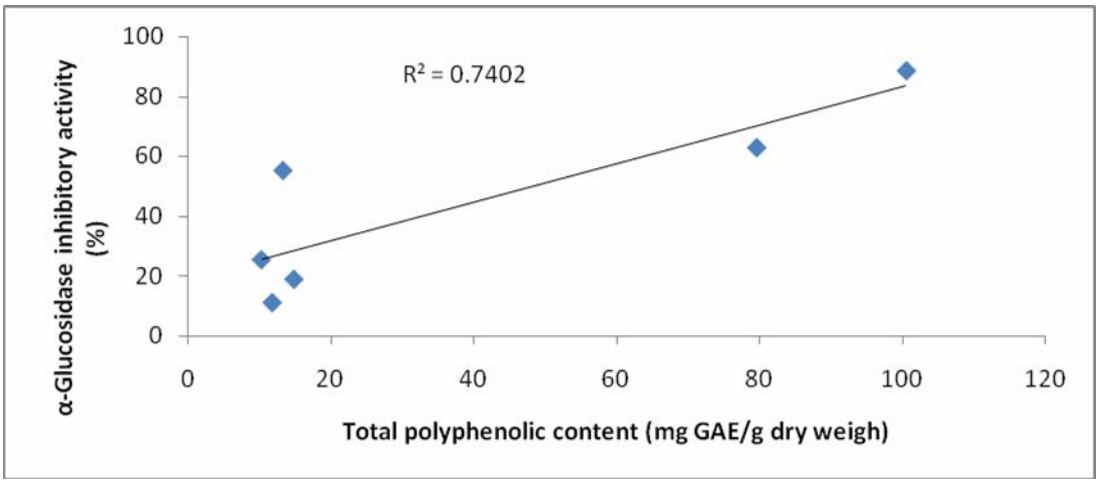


Figure 4-23The correlation between α-glucosidase inhibitory activities (%) and total polyphenolic contents (mg gallic acid equivalent/g dry weight of sample) of the fractions of the acetone extract of *F. lutea.*

4.2.6 Cytotoxicity of the fractions from acetone extract of *F. lutea*

The cytotoxic activities of the fractions against the Vero monkey kidney and the human C3A liver cell lines are presented in Table 4-7. In all assays, the hexane fraction was the least toxic (LD_{50} value >1000 µg/ml). The fractions were relatively more cytotoxic to the Vero kidney cells when compared to the C3A liver cells. The ethyl acetate fraction was relatively non-toxic although it had the lowest LD_{50} against the Vero cells (LD_{50} = 126.9 ± 1.5 µg/ml), while the n-butanol fraction was the most cytotoxic to the C3A liver cells with LC_{50} of 76.8 ± 0.4 µg/ml.

Table 4-7Cytotoxicity activity of fractions from extract of *F. lutea* (LC_{50} in µg/ml ± SE)

Fractions	Vero kidney cells	C3A liver cells
Hexane	□1000	□1000
chloroform	389.6 ± 1.8	615.7 ± 3.9
Dichloromethane	302.3 ± 2.1	□1000
Ethyl acetate	126.9 ± 1.5	□1000
n-Butanol	216.1 ± 2.9	76.8 ± 0.4
Water	ND	ND

Values are means of triplicate determinations done three times (n=9) ± standard error;

ND: not determined

4.2.7 Glucose uptake in C2C12 muscle cells

The effect of fractions of the acetone extract of *F. lutea* at different concentrations (15 µg/ml – 500 µg/ml) on glucose uptake in C2C12 muscle cells is presented in Figure 4-24. Of all the fractions, only the ethyl acetate and n-butanol fractions significantly (p□0.001) increased glucose uptake in the cells in a dose responsive manner with the ethyl acetate fraction enhancing the highest glucose uptake of 31.2 ± 1.5% at the highest concentration (500 µg/ml) followed by the n-butanol fraction enhancing glucose uptake of 25.9 ± 1.2% as the same concentration. The hexane, chloroform and dichloromethane fractions enhanced very low glucose uptake at only the lowest concentration (15 µg/ml).

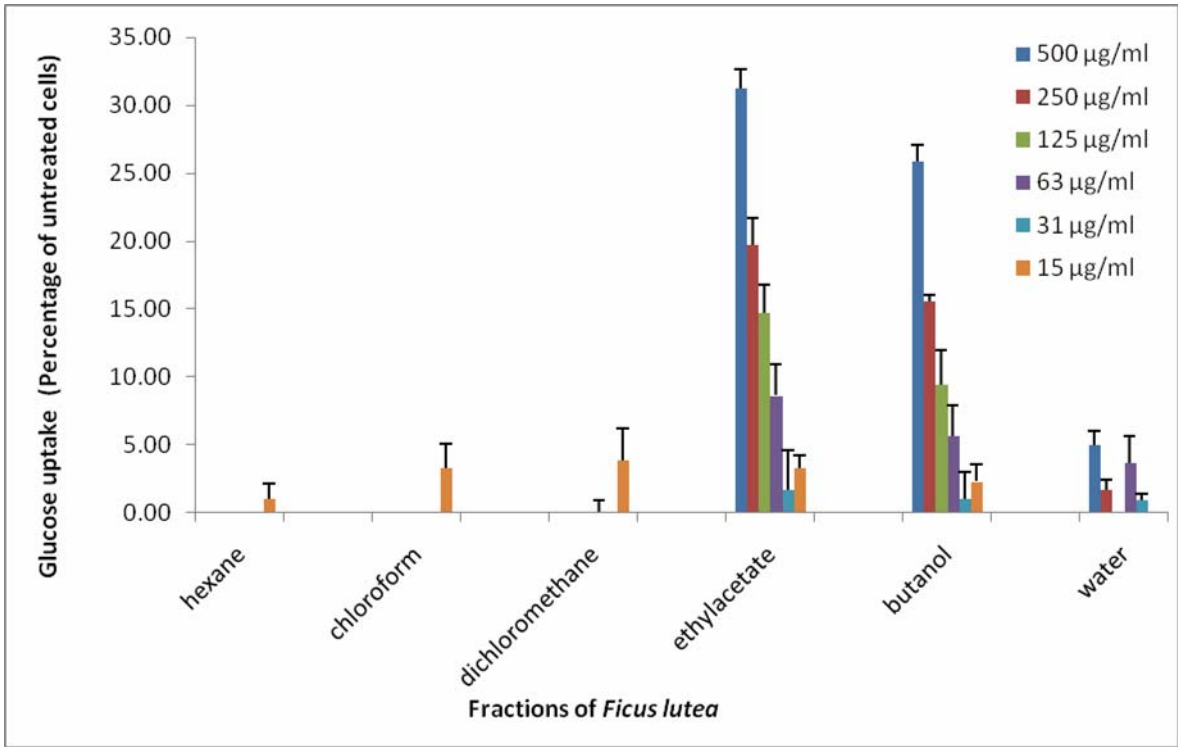


Figure 4-24Glucose uptake in C2C12 muscle cells (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the fractions of acetone extract of *F. lutea.*

With the ethyl acetate fraction significantly enhancing glucose uptake, the effect of concurrent insulin on glucose uptake in the presence of ethyl acetate fraction was investigated. The uptake of glucose by C2C12 cells treated with ethyl acetate fraction at different concentrations (15 µg/ml – 500 µg/ml) in medium containing two different concentrations of insulin (1 µM and 10 µM) is presented in Figure 4-25. The insulin-mediated glucose uptake of C2C12 exposed to the ethyl acetate fraction at different insulin concentrations of 1 µM and 10 µM was 26.8 ± 0.8% and 28.8 ± 0.7% respectively compared to ethyl acetate alone (31.2 ± 1.5%) at the highest ethyl acetate fraction concentration (500 µg/ml). Insulin at the two concentrations significantly (p□0.001) inhibited cell exposed to ethyl acetate from

increasing glucose uptake in comparison to ethyl acetate fraction in the absence of insulin. However since the difference was minor, the effect was not considered to be biologically significantly different.

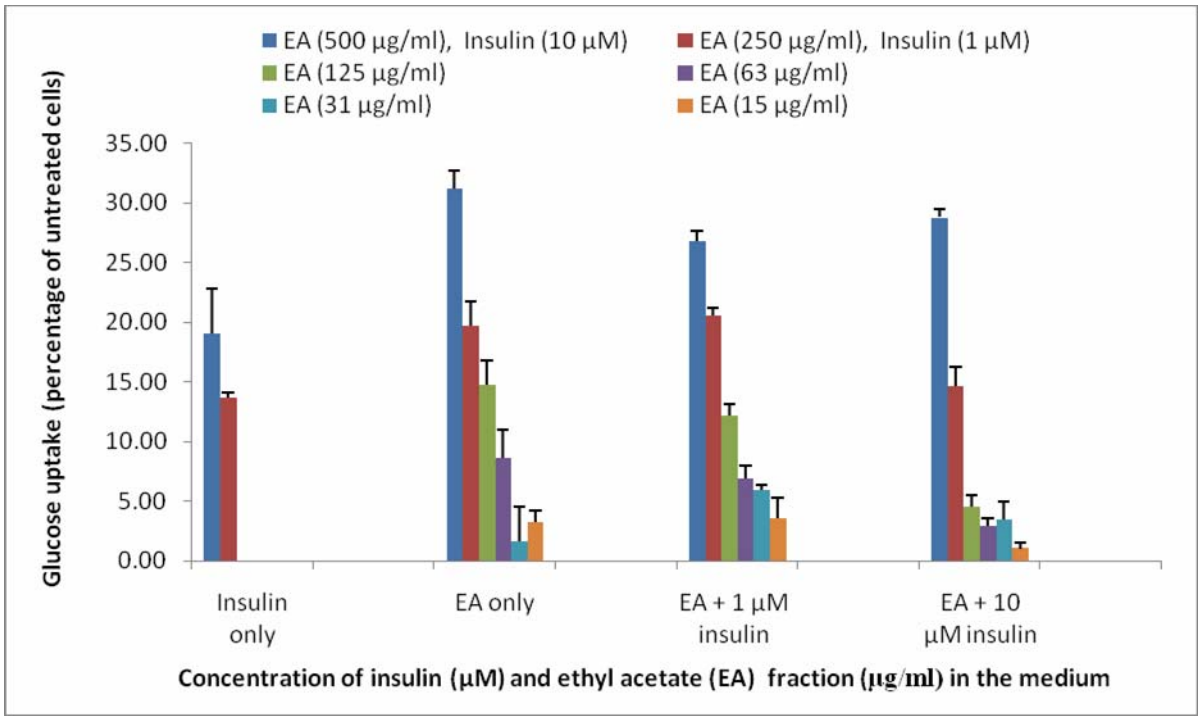


Figure 4-25Glucose uptake in C2C12 muscle cells (as percentage of untreated control cells ± standard error of mean, n=9) exposed to difference concentrations of the ethyl acetate fraction (represented by the different colours) in medium containing different concentrations of insulin (x-axis).

4.2.8 Glucose uptake activity in H-4-11-E liver cells

The effect of fractions at different concentrations (15 µg/ml – 500 µg/ml) on glucose uptake activity in H-4-11-E liver cells is presented in Figure 4-26. All fractions minimally enhanced glucose utilisation activity in H-4-11-E liver cells at the lowest concentration (15 µg/ml). Only the ethyl acetate and n-butanol significantly (p 0.001) increased glucose uptake in a dose responsive manner. The ethyl acetate fraction enhanced the highest (40.0 ± 2.8%) glucose uptake at the concentration of 500 µg/ml and this was followed by the n-butanol fraction (25.9 ± 1.4%) at the same concentration.

With the ethyl acetate fraction significantly enhancing glucose uptake activity, the effect of concurrent insulin on ethyl acetate mediated glucose uptake was evaluated. The effect of ethyl acetate fraction at different concentrations (15 µg/ml – 500 µg/ml) in medium containing two different concentrations of insulin (1 µM and 10 µM) on glucose uptake is presented in Figure 4-27. The insulin-mediated glucose uptake in H-4-II-E liver cells exposed to ethyl acetate fraction at different insulin

concentrations of 1 µM and 10 µM was 27.0 ± 0.8% and 29.5 ± 0.7% respectively when compared to ethyl acetate fraction alone (40.0 ± 2.8%) at the highest concentration (500 µg/ml).

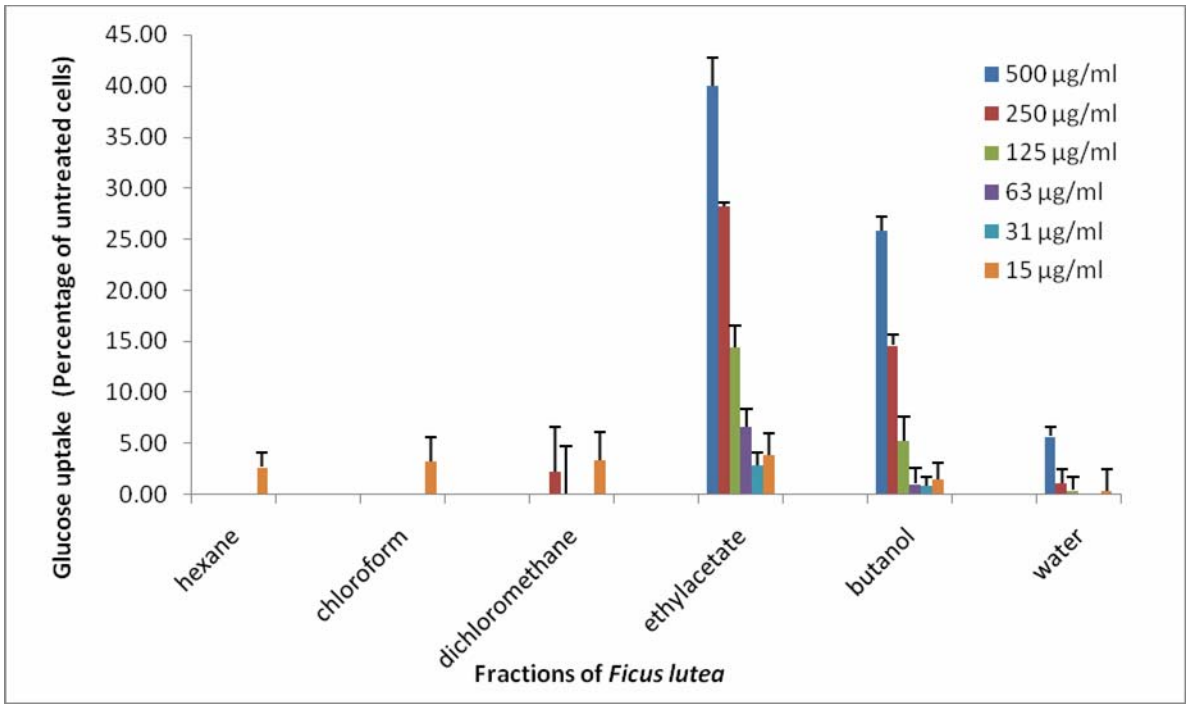


Figure 4-26Glucose uptake in H-4-11-E rat liver cells (as percentage of untreated control cells ± standard error of mean, n=9 for) exposed to the fractions of acetone extract of *F. lutea*.

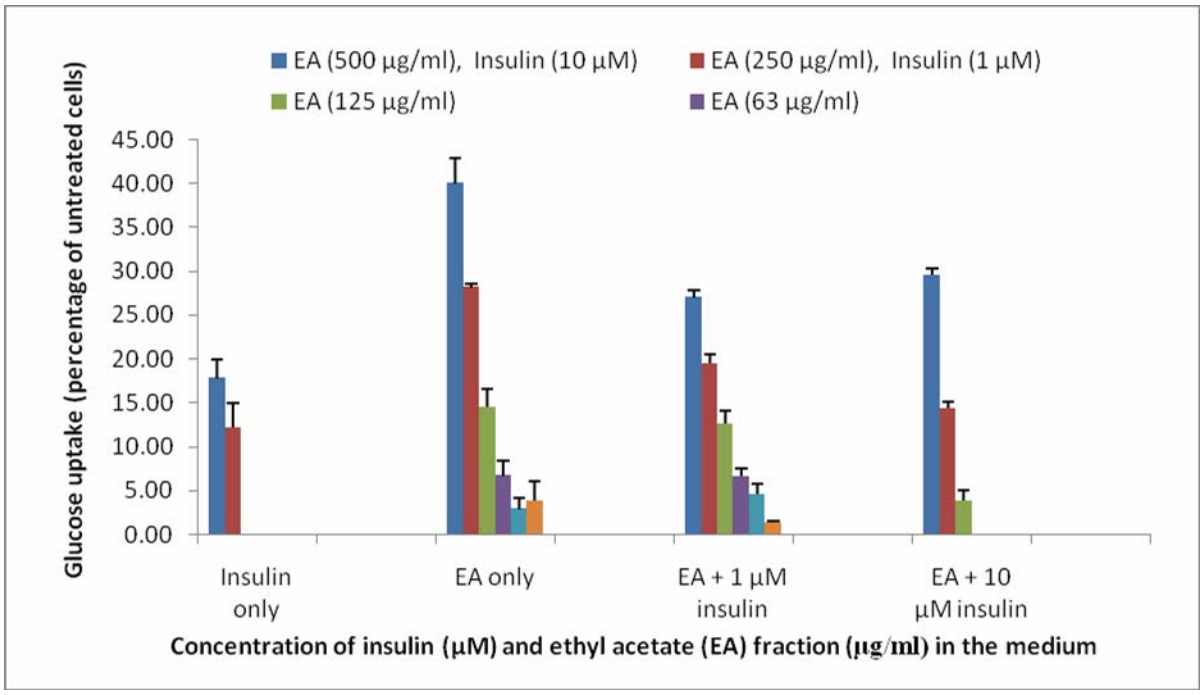


Figure 4-27Glucose uptake in H-4-11-E rat liver cells (as percentage of untreated control cells ± standard error of mean, n=9) exposed to different concentration of the ethyl acetate fraction (different colours) in medium containing different concentrations of insulin (x-axis).

4.2.9 Insulin secretion in RIN-m5F pancreatic cells

With the ethyl acetate fraction of *F. lutea* being the most active fraction in stimulating glucose uptake in the treated C2C12 muscle and H-4-II-E cells, the effect of this fraction at different concentrations (62.5 µg/ml – 500 µg/ml) on insulin secretion was investigated in RIN-m5F pancreatic β-cells and was compared with the untreated control cells. The RIN-m5F pancreatic cells exposed to the ethyl acetate fraction resulted in a dose related increase in insulin secretion (Figure 4-28). The insulin releasedsignificantly (p□0.001) increased from 33.0 ± 7.3% at the concentration of 62.5 µg/ml to 115.0 ± 4.4% at the concentration of 250 µg/ml. The 500 µg/ml dose has no superior response to the 250 µg/ml dose, indicating that the maximum effect has plateaued.

The effect of the ethyl acetate fraction of *F. lutea* on the viability of RIN-m5F pancreatic β-cells after insulin secretion assay was investigated (Figure 4-29). The result showed that the RIN-m5F cells exposed to the ethyl acetate fraction had dose related decrease in cell viability from 91.9 ± 8.6% to 50.2 ± 2.8% as concentration was increased from 62.5 µg/ml to 500 µg/ml. The correlation coefficient between the viability of RIN-m5F pancreatic β-cells and insulin secretion by the ethyl acetate fraction of the extract of *F. lutea* R^2 was 0.76 (Figure 4-30). This indicates that the stimulation of RIN-m5F pancreatic β-cells by the ethyl acetate fraction of the extract of *F. lutea* to secrete insulin may in part be attributed to the disruption of cell membrane.

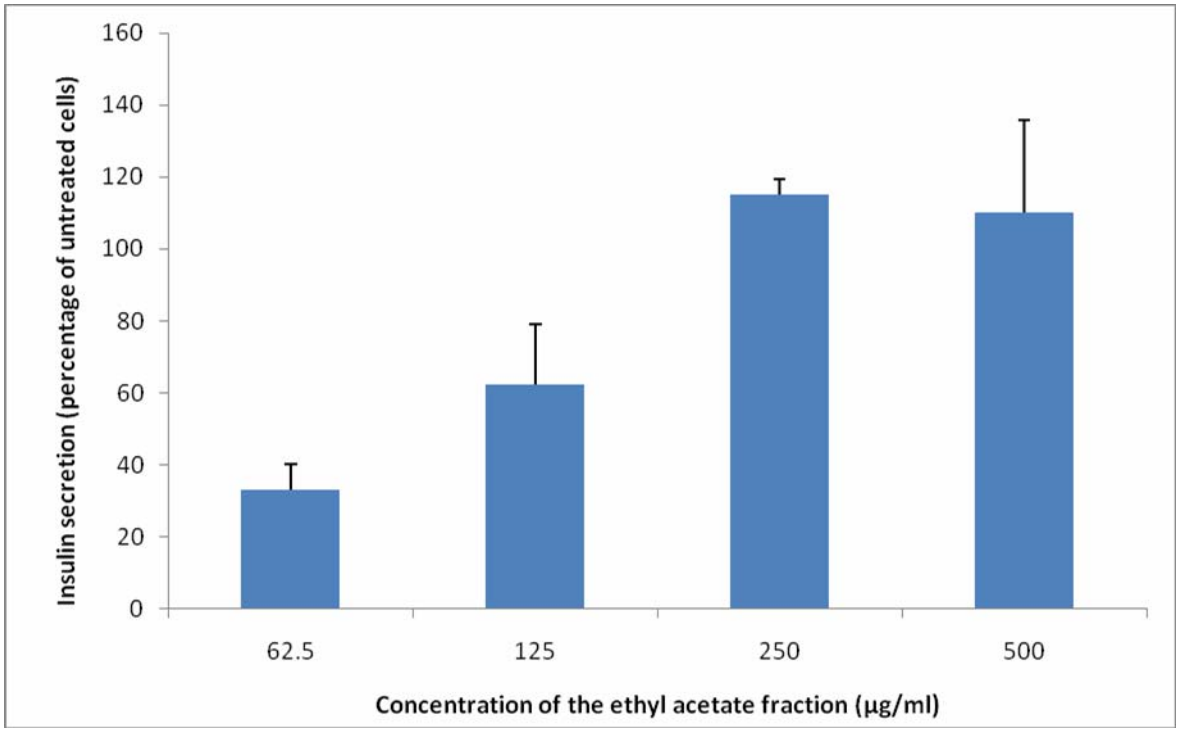


Figure 4-28Insulin secreted in RIN-m5F pancreatic cells (as percentage of untreated control cells ± standard error of mean, n=6) exposed to the ethyl acetate fraction from crude acetone extract of *F. lutea.*

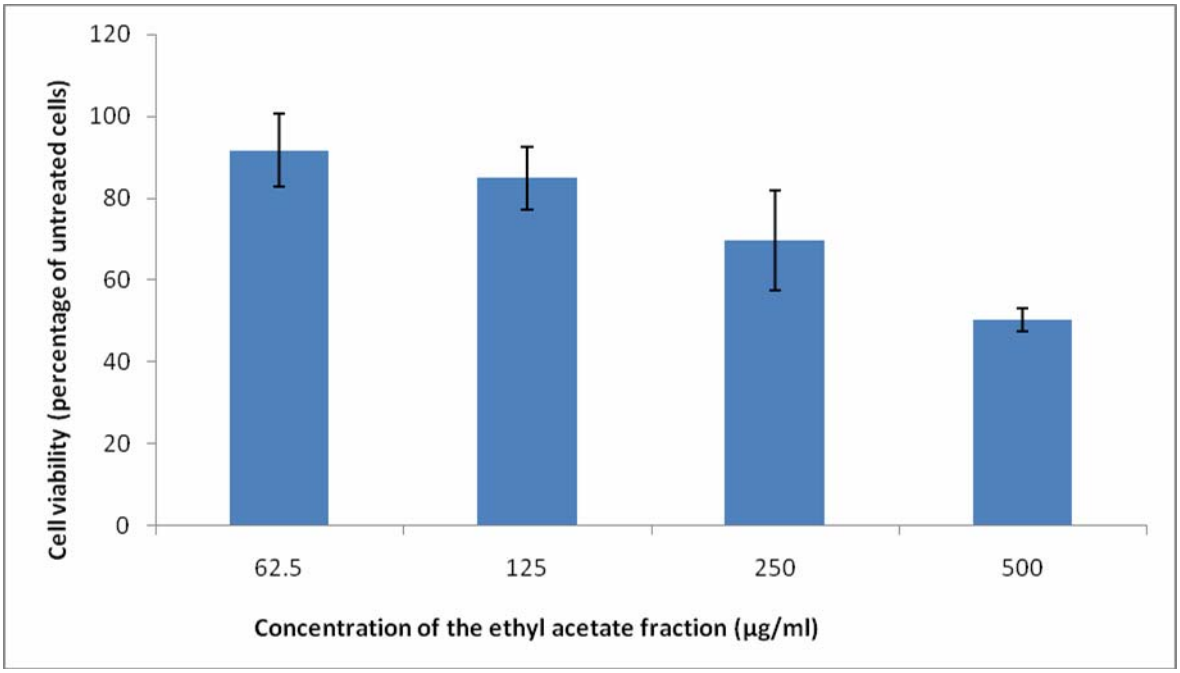


Figure 4-29Effect of the ethyl acetate fraction from the extract of *F. lutea* on RIN-m5F pancreatic cell viability (as percentage of untreated control cells ± standard error of mean, n=6) after stimulating insulin secretion.

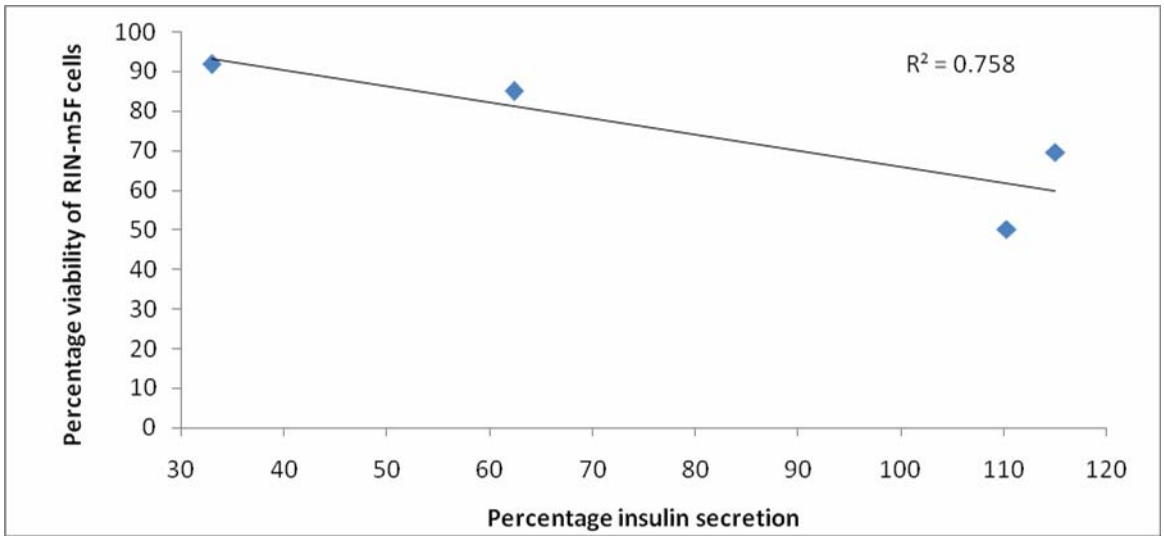


Figure 4-30 The correlation between percentage cell viabilty of RIN-m5F pancreatic β-cells and percentage insulin secretion by the ethyl acetate fraction of the extract of *F. lutea*.

4.3 Structure Elucidation of Compounds from the ethyl acetate fraction of F. lutea

4.3.1 Structure Elucidation of AFL1 or Lupeol (1)

AFL1 was obtained as white powder. Its ^{1}H and ^{13}C NMR spectra (Figure 1 and 2 in Appendix) exhibited signals at δ 4.68 (*brs*, H-29_a), 4.47 (*brs*, H-29_b), 3.18 (*m*, H-3), 109.4 (C-29), and 79.1 ppm (C-3) assignable respectively to protons and carbons of a methylene and oxymethine groups at positions 29 and 3 of a lup-20(29)-en-3-ol class of triterpenes (Mahato and Kundu, 1994). Furthermore, one characteristic signal of carbon C-20 of lupeol structure was observed on ^{13}C NMR spectrum at δ 148.2 ppm while seven singlet signals relevant for angular methyl groups appeared on the ^{1}H NMR spectrum between 0.8-1.7 ppm. All the NMR data including ^{1}H (Figure 1 in Appendix), ^{13}C (Figure 2 in Appendix), ^{1}H ^{1}H COSY (Figure 3 in Appendix), HSQC (Figure 4) and HMBC (Figure 5 in Appendix) were in agreement with those published for lupeol (Figure 4-31) previously isolated from the same species (Poumale *et al.*, 2011).

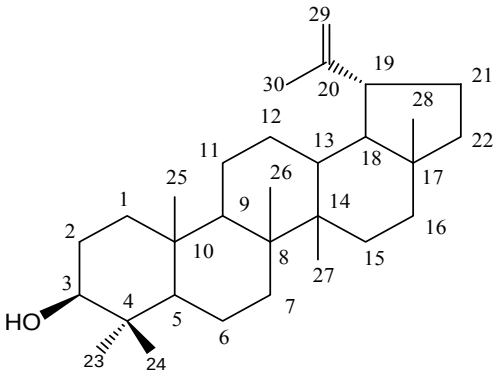


Figure 4-31The structure of AFL1 (lupeol) isolated from the leaves of *F. lutea*.

4.3.2 Structure Elucidation of AFL2 or Stigmasterol (2)

AFL2 was obtained as a white powder. The presence of total number of 30 carbons on its ^{13}C NMR spectrum (Figure 6 in Appendix) including signals due to six angular methyl groups was relevant for this compound to be a phytosterol. The ^{13}C NMR spectrum displayed signals at δ 140.7, 138.3, 129.2, 121.7 and 71.8 ppm corresponding respectively to carbons C-5, C-22, C-23, C-6 and C-3 of stigmasterol structure. The ^{1}H NMR spectrum (Figure 7 in Appendix) exhibited signals at δ 5.32 (*brd*, 2.2; 3.0 Hz, 1H), 5.17 (*dd*, 8.5 Hz, 1H), 5.00 (*dd*, 8.5 Hz, 1H) and 3.50 (*m*, 1H) corresponding to protons H-6, H-22, H-23 and H-3 respectively. Furthermore, signals due to methyl groups were observed on its ^{1}H NMR between 0.90 and 0.60 ppm. The ^{1}H and ^{13}C NMR (Figure 6 and 7 in Appendix) as well as the ^{1}H ^{1}H COSY (Figure 8 in Appendix), HSQC (Fig 9 in Appendix) and HMBC (Figure 10 in Appendix) data were in agreement with those of previously reported stigmasterol (Figure 4-32) ((Forgo and Kövér, 2004). This compound (Figure 4-32) which is very common in plant kingdom is isolated for the first time from this species.

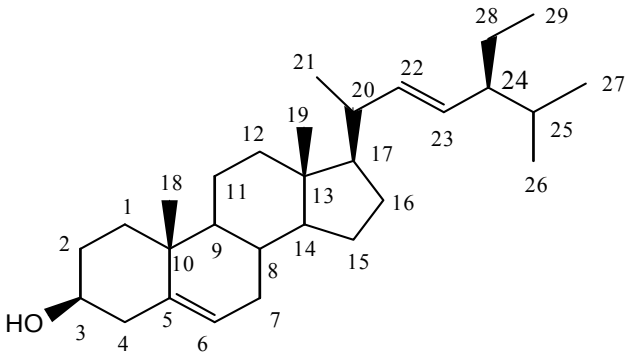


Figure 4-32The structure of compound AFL2 (stigmasterol) isolated from the leaves of *F. lutea.*

4.3.3 Structure Elucidation of AFL3 or α-Amyrin Acetate (3)

AFL3 was obtained as oil. Its ^{13}C NMR spectrum (Figure 11 in Appendix) exhibited a total number of 30 carbons with 4 characteristic downfields displayed at δ 170.9, 139.7, 124.4 and 81.0 assignable to a triterpene skeleton with one carbonyl acetate (CH_3CO), one ethylenic double bond (C=CH) and one oxymethine (HCOH) groups. The ^{1}H NMR spectrum (Figure 12 in Appendix) exhibited characteristic signals at δ 5.12 (*t*, 3.7 Hz, 1H), 4.49 (*m*, 1H) and 2.02 (*s*, 3H) due to protons at positions C-12, C-3 and acetyl group from α-amyrin acetate (Mahato and Kundu, 1994). All these data above as well as DEPT (Figure 13 in Appendix), ^{1}H ^{1}H COSY (Figure 14 in Appendix), HSQC (Figure 15 in Appendix) and HMBC (Figure 16 in Appendix) were similar to those published for α-amyrin acetate (Figure 4-33) previously isolated from the same species (Poumale *et al.*, 2011).

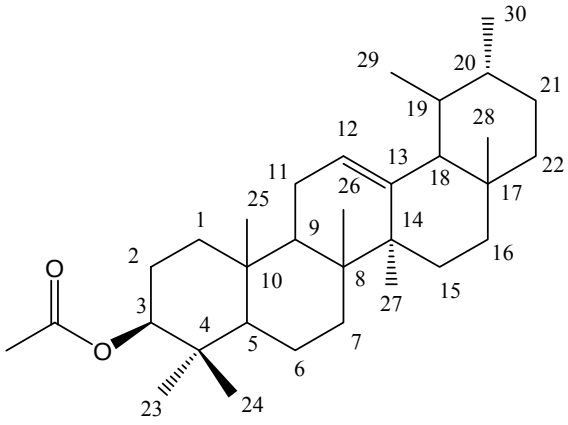


Figure 4-33The structure of compound AFL3 (α-amyrin acetate) isolated from the leaves of *F. lutea*.

4.3.4 Structure elucidation of AFL4 or Epicatechin (4)

AFL4 was obtained as a yellowish powder. Its ^{1}H NMR spectra (Figure 17 in Appendix) showed singlets at δ 8.10, 7.94, 7.79 and 7.74 ppm assignable to four phenolic protons. The ^{1}H NMR spectrum also exhibited a broad singlet, one multiplet and two doublet of doublet signals at δ 4.87 (*brs*, 1H), 4.20 (*m*, 1H), 2.85 (*dd*, 4.6, 16.5 Hz, 1H) and 2.72 (*dd*, 3.3, 16.7 Hz, 1H) attributable to protons H-2, H-3, H-4_b and H-4_a, respectively. Furthermore, the ^{1}H NMR spectrum showed two sets of aromatic protons: the first one at δ 6.01 (*d*, 2.3 Hz, H-6) and 5.91 (*d*, 2.3 Hz, H-8) corresponding to A ring, and the second one at δ 7.04 (*d*, 2.0 Hz, H-2'), 6.88 (*d*, 8.4 Hz, H-5') and 6.83 ppm (*dd*, 2.0, 8.4 Hz, H-6') imputable to B ring protons. The ^{13}C NMR spectrum (Figure 18) exhibited the characteristic flavan-3-ol signals at δ 79.4, 66.9 and 28.9 ppm corresponding to C-2 (OCH), C-3 (COH) and C-4 (CH_2), respectively (Morimoto *et al.*, 1985; Pan *et al.*, 2003). Moreover, the ^{13}C NMR spectrum exhibited 12 aromatic carbons at δ 145.2, 145.3, 157.1, 157.5 and 157.6 ppm attributable respectively to five oxygenated carbons C-3', C-4', C-5, C-7 and C-9, and at δ 132.2, 115.2, 115.4, 119.3, 96.1, 96.0 and 99.8 corresponding to carbons C-1', C-2', C-5', C-6', C-6, C-8 and C-10, respectively. Unambiguous assignments for the ^{1}H and ^{13}C NMR for this compound were made by the combination of DEPT (Figure 19 in Appendix), ^{1}H-^{1}H COSY (Figure 20 in Appendix), HSQC (Figure 21 in Appendix) and HMBC (Figure 22 in Appendix) spectra. All the spectroscopic data above were in agreement with those reported for epicatechin (Figure 4-34) previously isolated and characterized by Jiang and co-workers from pericarp tissues of lychee fruit (Zhao *et al.*, 2006). The isolation of this compound is reported here for the first time from this species.

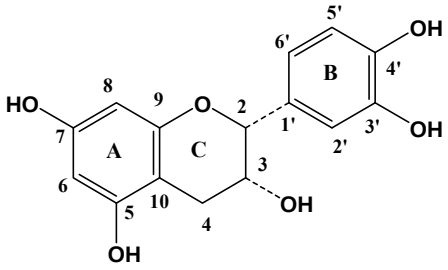


Figure 4-34The structure of AFL4 (epicatechin) isolated from the leaves of *F. lutea*.

4.3.5 Structure elucidation of AFL5 or Epiafzelechin (5)

AFL4 was obtained as yellowish powder. Its ^{13}C NMR and DEPT spectra (Figure 23 and 24 in Appendix) exhibited signals at δ 78.7, 66.1 and 28.1 ppm corresponding to carbons C-2, C-3 and C-4 characteristic for a flavan-3-ol skeleton. This was confirmed by the presence of important signals on its ^{1}H NMR spectrum (Figure 24 in Appendix) at δ 4.87 (*brs*, H-2), 4.15 (*m*, H-3), 2.80 (*dd*, 4.4, 16.8 Hz, H-4$_a$) and 2.62 (*dd*, 3.4, 16.6 Hz, H-4$_b$) due to protons at positions C-2, C-3 and C-4, respectively. Two sets of aromatic protons were observed on its ^{1}H NMR spectrum: the first set appeared as two doublets at δ 5.98 (*d*, 2.2 Hz, H-8) and 5.87 (d, 2.2 Hz, H-6) due to the ring-A while the second one appeared as AA'BB' system at δ 7.28 (*d*, 8.4 Hz, H-2'/H-6') and 6.76 (*d*, 8.4 Hz, H-3'/H-5') corresponding to B-ring. Furthermore, the ^{1}H NMR spectrum also displayed three downfield broad signals between 9.5 and 8.9 ppm assignable to three hydroxyl groups at C-4', C-7 and C-5. The broad singlet multiplicity of proton H-2 was indicative to this proton to be in *cis* configuration with proton H-3. Moreover, the ^{13}C NMR spectrum exhibited signals at δ 156.9, 156.2, 130.4 and 99.0 ppm corresponding to carbons C-7/C-4', C-5/9, C-1' and C-10, respectively. All these data were in agreement with those published for epiafzelechin (Figure 4-35) (Kpegba *et al.*, 2010). This compound is isolated here for the first time from this species.

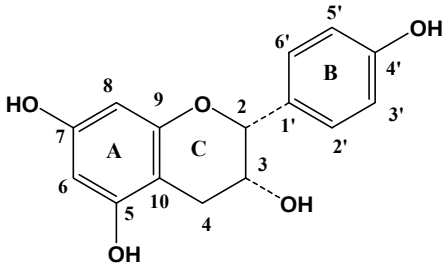


Figure 4-35The structure of AFL5 (epiafzelechin) isolated from the leaves of *F. lutea*.

4.3.6 Inhibition of α-glucosidase activity by the compounds

The concentration of the compounds isolated from the ethyl acetate fraction of *F. lutea* leading to inhibition of 50% of α-glucosidase (sucrase) activity (EC_{50}) is presented in Table 4-8. Epicatechin was the most potent with an EC_{50} value of 5.72 ± 2.7 µg/ml and this was followed by epiafzelechin (EC_{50} value = 7.64 ± 37.5 µg/ml), with no significant difference between them. The lupeol was the least active (EC_{50}>1000) among the compounds isolated.

Table 4-8The EC_{50} sucrase activity of compounds from ethyl acetate fraction of *F. lutea*

Compound	EC_{50} (µg/ml)
Lupeol	>1000
Stigmasterol	115.71 ± 11.6[a]
α-Amyrin acetate	335.82 ± 22.6[a]
Epicatechin	5.72 ± 2.6[b]
Epiafzelechin	7.64 ± 4.9[b]

[a,b]No significant difference between extracts with same value, but significant difference p□0.05 between different values.

4.3.7 Glucose uptake in C2C12 muscle cells

The effect of the compounds isolated from the ethyl acetate fraction of *F. lutea* at different concentrations (15 µg/ml – 250 µg/ml) on glucose uptake in C2C12 muscle cells is presented in Figure 4-36. Of the five isolated compounds only epicatechin and epiafzelechin significantly (p□0.001) enhanced glucose utilisation activity in C2C12 muscle. Epiafzelechin increased glucose uptake in a dose responsive manner with the highest uptake (33.4 1.8%) resulting at a concentration of 250 µg/ml. Similarly epicatechin increased glucose uptake in a concentration dependent manner with the highest uptake of 40.7 ± 1.9% at 63 µg/ml.

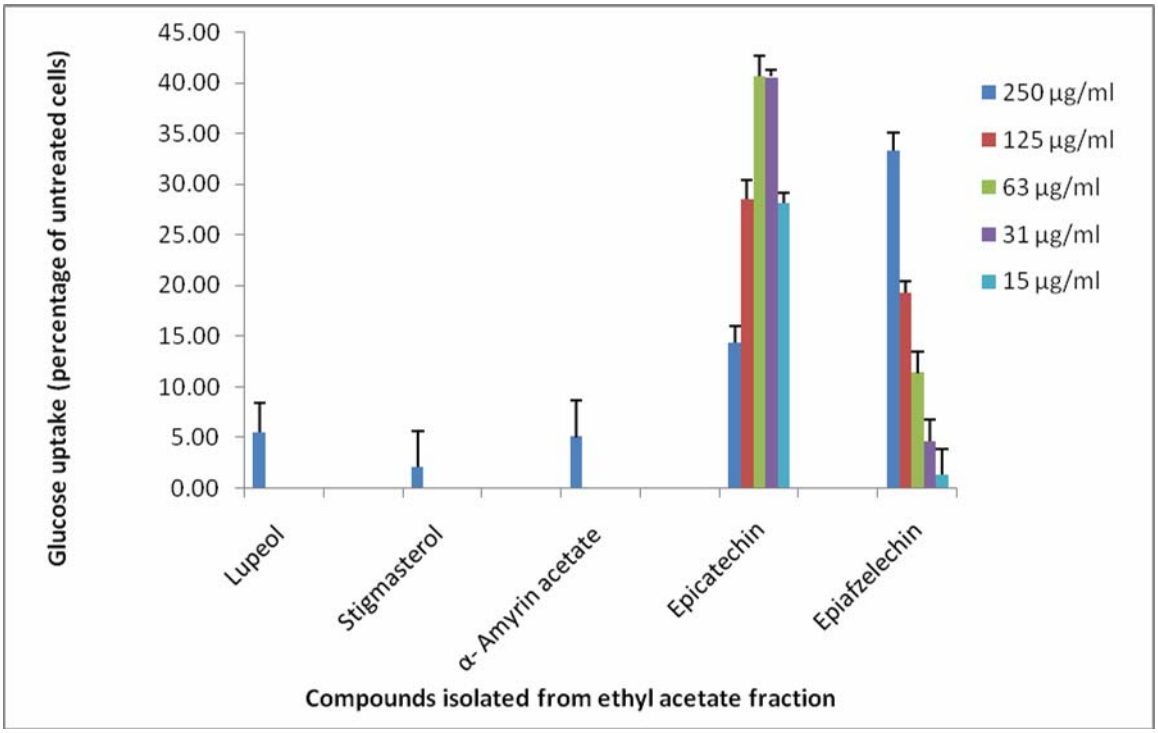


Figure 4-36Glucose uptake in C2C12 muscle cells (as percentage of untreated cells control cells ± standard error of mean, n=9) exposed to the fractions of acetone extract of *F. lutea.*

The effect of epiafzelechin on glucose uptake by the muscle cells, in the presence of insulin was subsequently evaluated. Epicatechin was not evaluated due to time constraints. The uptake of glucose by C2C12 cells treated with epiafzelechin at different concentrations (15 µg/ml – 250 µg/ml) in medium containing twodifferent concentrations of insulin (1 µM and 10 µM) is presented in Figure 4-37. The insulin-mediated glucose uptake of C2C12 exposed to epiafzelechin at different insulin concentrations of 1 µM and 10 µM was 33.2 ± 0.5% and 34.5 ± 1.1% respectively compared to epiafzelechin alone (33.4 ± 1.8%) at the highest epiafzelechin concentration (250 µg/ml).

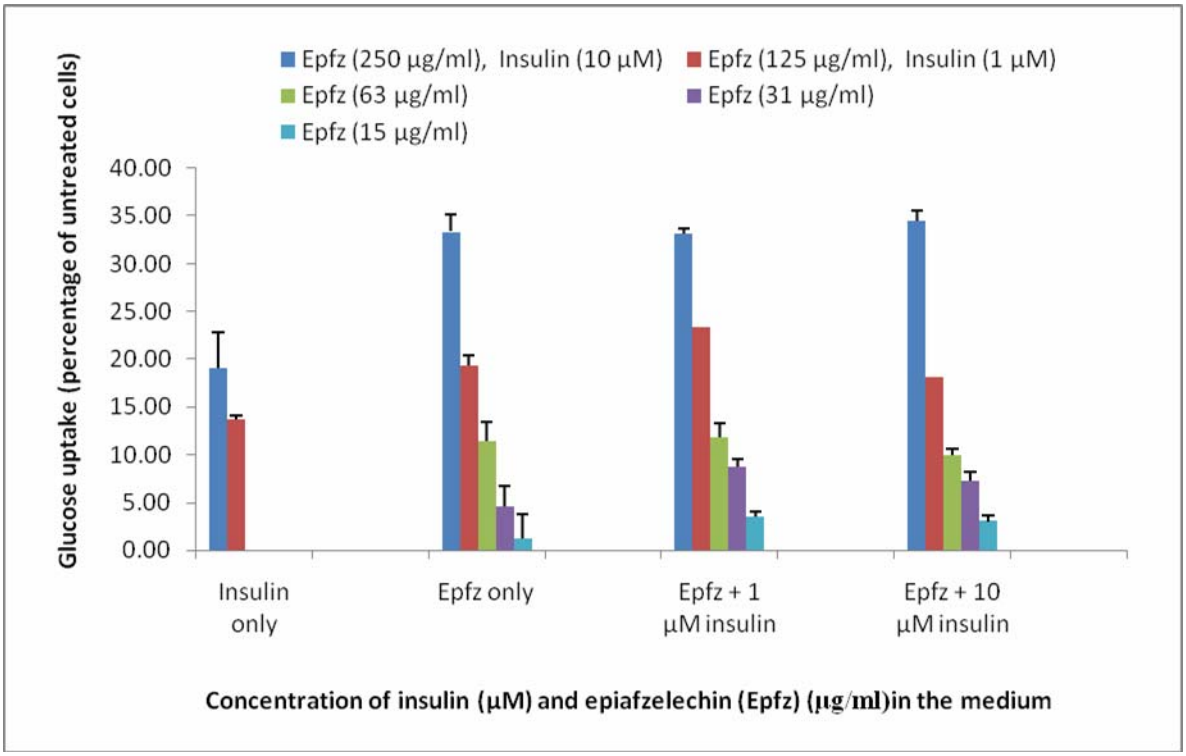


Figure 4-37Glucose uptake in C2C12 muscle cells (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the epiafzelechin at different concentrations (represented by the different colours) in medium containing different concentrations of insulin (on the x-axis).

4.3.8 Glucose uptake in H-4-11-E liver cells

The effect of the compounds isolated from the ethyl acetate fraction of *F. lutea* at different concentrations (15 µg/ml – 250 µg/ml) on glucose uptake in H-4-II-E liver cells is presented in Figure 4-38. Of the five isolated compounds only epicatechin and epiafzelechin significantly (p□0.001) increased glucose uptake in a concentration dependent manner with epicatechin enhancing glucose uptake of 46.7 ± 1.2% at the concentration of 250 µg/ml and this was followed by epiafzelechin with an uptake of 32.4 ± 1.5% at the same concentration.

The effect of epiafzelechin on glucose uptake by the H-4-II-E liver cells, in the presence of insulin was subsequently evaluated. The uptake of glucose by C2C12 cells treated with epiafzelechin at different concentrations (15 µg/ml – 250 µg/ml) in medium containing two different concentrations of insulin (1 µM and 10 µM) is presented in Figure 4-39. Epicatechin was not evaluated due to time constraints. The insulin-mediated glucose uptake in H-4-II-E liver cells exposed to epiafzelechin at different insulin concentrations of 1 µM and 10 µM was 36.9 ± 1.0% and 37.9 ± 0.9% respectively when compared to epiafzelechin alone (32.4 ± 1.5%) at the highest concentration (250 µg/ml).

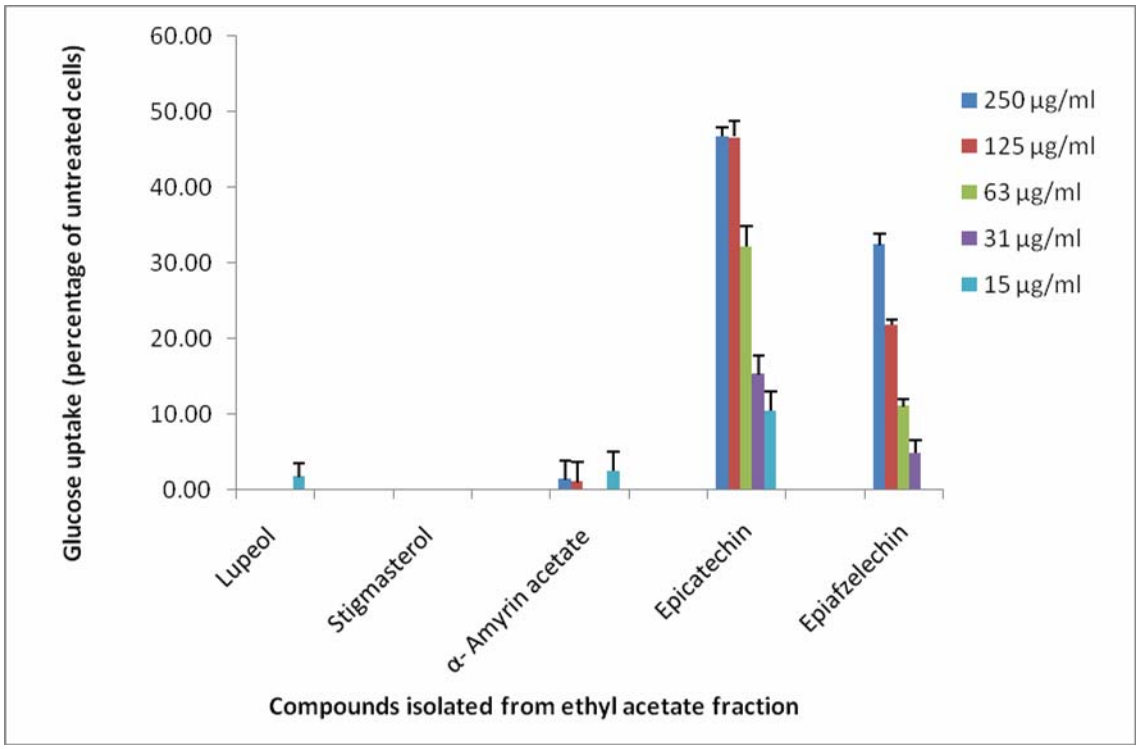


Figure 4-38Glucose uptake in H-4-11-E rat liver cells (as percentage of untreated control cells ± standard error of mean, n=9 for) exposed to the fractions of acetone extract of *F. lutea*.

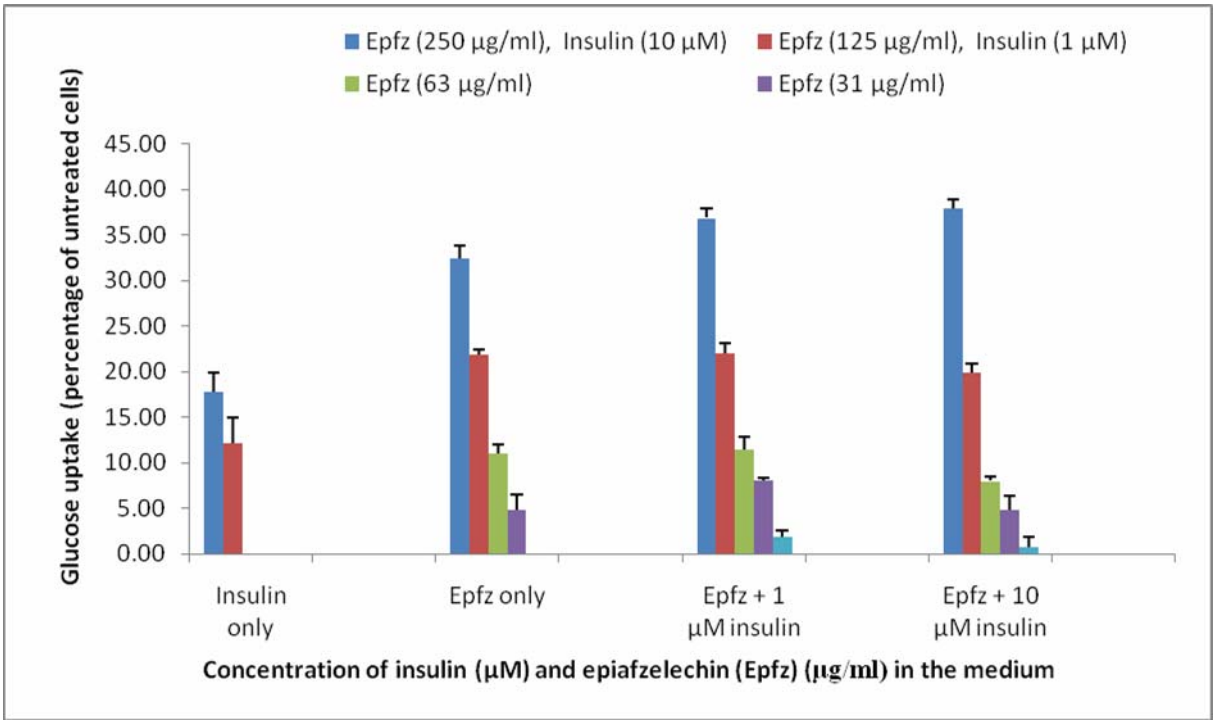


Figure 4-39Glucose uptake in H-4-II-E rat liver cells (as percentage of untreated control cells ± standard error of mean, n=9) exposed to the compound epiafzelechin in medium containing different concentrations of insulin.

4.3.9 Insulin secretion in RIN-m5F pancreatic cells

Epiafzelechin was evaluated at different concentrations (62.5 µg/ml – 500 µg/ml) for its ability to stimulate insulin secretion in RIN-m5F pancreatic β-cells and was compared with the untreated control cells. Epicatechin was not evaluated due to time constraints. The RIN-m5F pancreatic cells exposed to the epiafzelechin resulted in a dose related increase in insulin secretion (Figure 4-40). The insulin secreted significantly (p□0.001) increased from 47.1 ± 10.2% at the concentration of 62.5 µg/ml to 123.9 ± 19.2% at the concentration of 500 µg/ml.

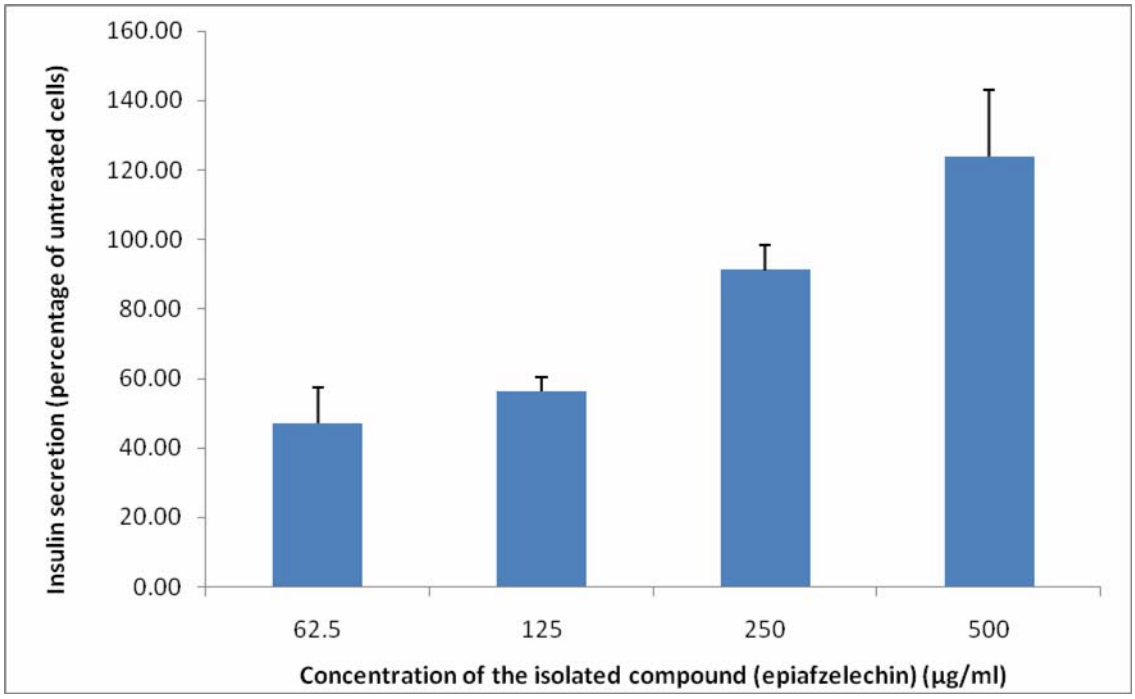


Figure 4-40Insulin secreted in RIN-m5F pancreatic cells (as percentage of untreated control cells ± standard error of mean, n=6) exposed to the isolated compound (epiafzelechin).

The effect of the isolated compound (epiafzelechin) on the viability of RIN-m5F pancreatic β-cells after insulin secretion assay was investigated (Figure 4-41). The result showed that the RIN-m5F cells exposed to epiafzelechin had dose related decrease in cell viability from 106.4 ± 1.6% to81.1 ± 0.3% as concentration was increased from 62.5 µg/ml to 500 µg/ml. The correlation coefficient between the viability of RIN-m5F pancreatic β-cells and insulin secretion by the ethyl acetate fraction of the extract of *F. lutea* R^2 was 0.66 (Figure 4-42). This indicates that the stimulation of RIN-m5F pancreatic β-cells by epiafzelechin to secrete insulin may be attributed in part to disruption of cell membrane.

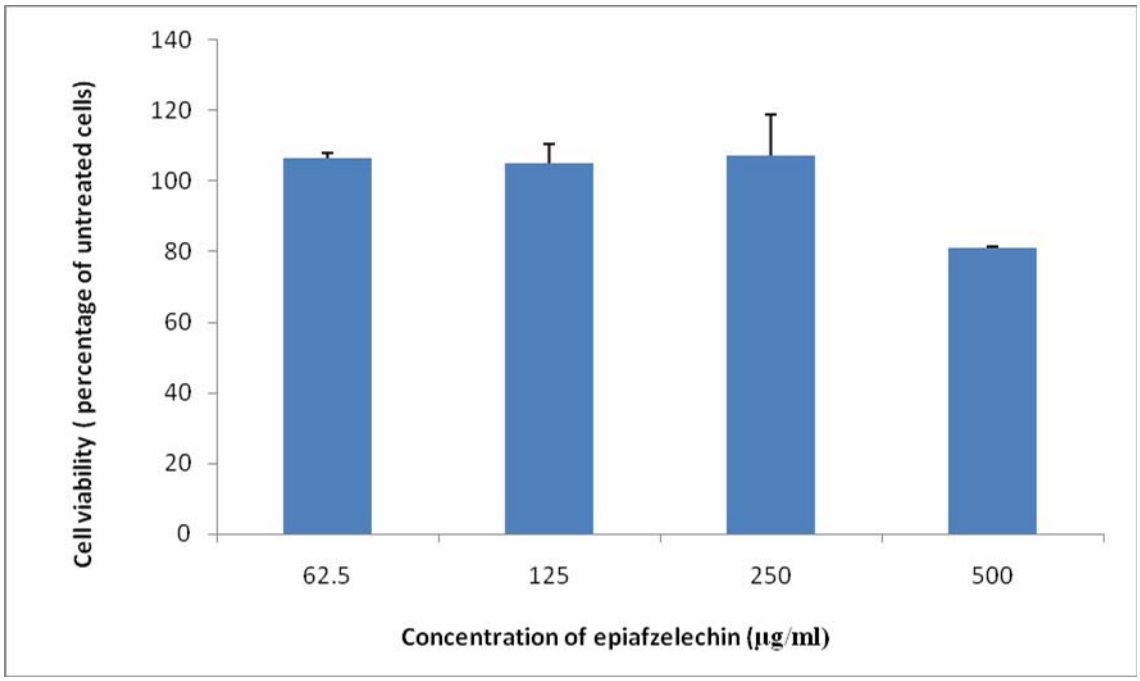


Figure 4-41Effect of epiafzelechin on RIN-m5F pancreatic cell viability (as percentage of untreated control cells± standard error of mean, n=6) after stimulating insulin secretion.

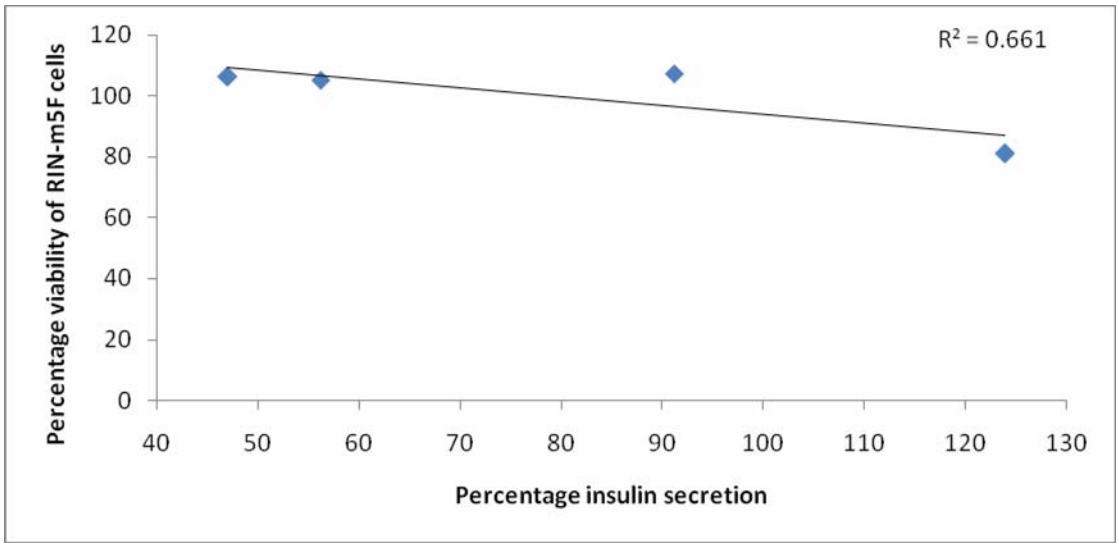


Figure 4-42 The correlation between percentage cell viabilty of RIN-m5F pancreatic β-cells and percentage insulin secretion by epiafzelechin.

4.4 *In vivo* study

4.4.1 The effect of different diets on body weight

Prior to the start of treatments, four mice died from the high caloric diet. Histological evaluation of the mice indicated that they died from ulcerative dermatitis. This condition is known to occur in mice fed a high fat diet most likely due to deficiency in the antioxidant vitamins. Due to the prior allocation of animals to treatment groups, the study was unbalanced with all treatment groups having ten animals, with the exception of the high caloric diet treatment group which has 6 miceThe initial weights of the CD1 mice (mean ± S.E.M.) at period 0 (Figure 4-43) before commencement of treatment was 40.45 ± 2.35 g (high calorie diet control), 41.02 ± 1.12 g (high calorie diet with treatment), 44.77 ± 0.48 g (normal diet control) and 42.39 ± 1.88 g (normal diet with treatment). The mice were hereafter fed their respective diet for about 7 weeks and the result of the different diet on body weight is presented in Figure 4-43. The body weight of the control mice fed the high calorie diet and the normal diet gradually increased throughout the study more than that of their comparative treatment groups, with the high calorie diet having the greater increase. Conversely, the body weight of mice on treatment in conjunction with a high calorie diet and normal diet with (ethyl acetate fraction) showed a gradual decrease in body weight throughout the study, with the mice on normal diet having the greatest reduction. Unfortunately none of these differences were significant ($p \square 0.05$) between the treatments and their controls. Similarly there was no significant difference ($p \square 0.05$) between the two treatment groups (high calorie diet with treatment group and the normal diet with treatment group) nor between the untreated groups (high calorie diet group and normal diet group). The final weight of mice at the end of the treatment plan was 44.14 ± 4.67 g (high calorie diet group), 40.88 ± 0.92 g (high calorie diet with treatment group), 45.90 ± 2.10 g (normal diet group) and 39.54 ± 1.34 (normal diet with treatment group).

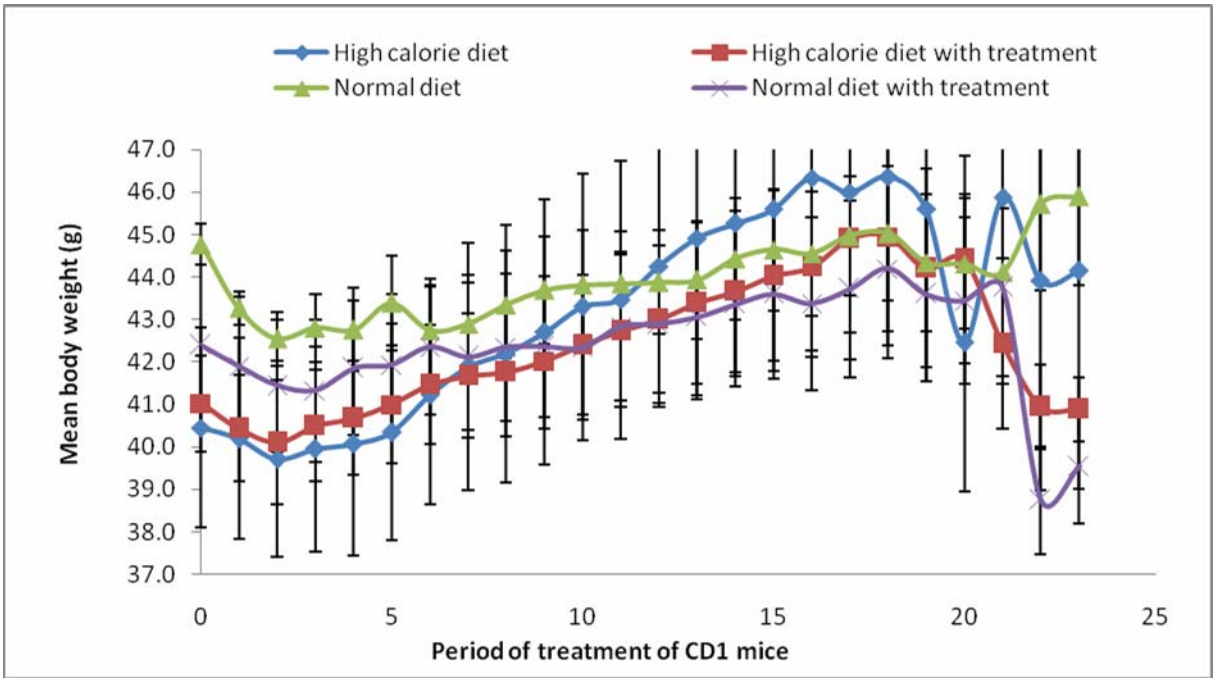


Figure 4-43The effect of high calorie and normal diet with and without treatment (the ethyl acetate fraction of *F. lutea*) on body weight of CD1 mice (mean ± S.E.M.). The initial body weight at period 0 was when obesity state was attained by mice prior to commencement of treatment for about 7 weeks.

4.4.2 The effect of different diets on food intake

After induction of obesity the mean food intake before commencement of treatment was 9.50 ± 1.42 g (high calorie diet group), 9.40 ± 1.01 g (high calorie diet with treatment group), 10.50 ± 1.12 g (normal diet group) and 10.60 ± 1.20 g (normal diet with treatment group). The mice were fed their respective diet for about 7 weeks and the result of food intake is presented in Figure 4-44. The mice placed on normal diet with and without treatment (ethyl acetate fraction of *F. lutea*) ate more food than those on high calorie diet with and without treatment. The food intake in mice on normal diet group was not significantly different (p□0.05) from that of mice placed on normal food with treatment. Similarly, food intake in mice placed on high calorie diet was not significantly different from those on high calorie with treatment throughout the study. Furthermore, there was no significant difference between the food intake in mice fed normal diet with treatment and mice fed high calorie diet with treatment, nor was there any significant difference between there controls. The Final food intake at the end of the study was 10.60 ± 1.07g (high calorie diet group), 9.00 ± 0.98 g (high calorie diet with treatment group), 14.00 ± 0.53 g (for the normal diet group) and 12.20 ± 0.98 g (normal diet with treatment group).

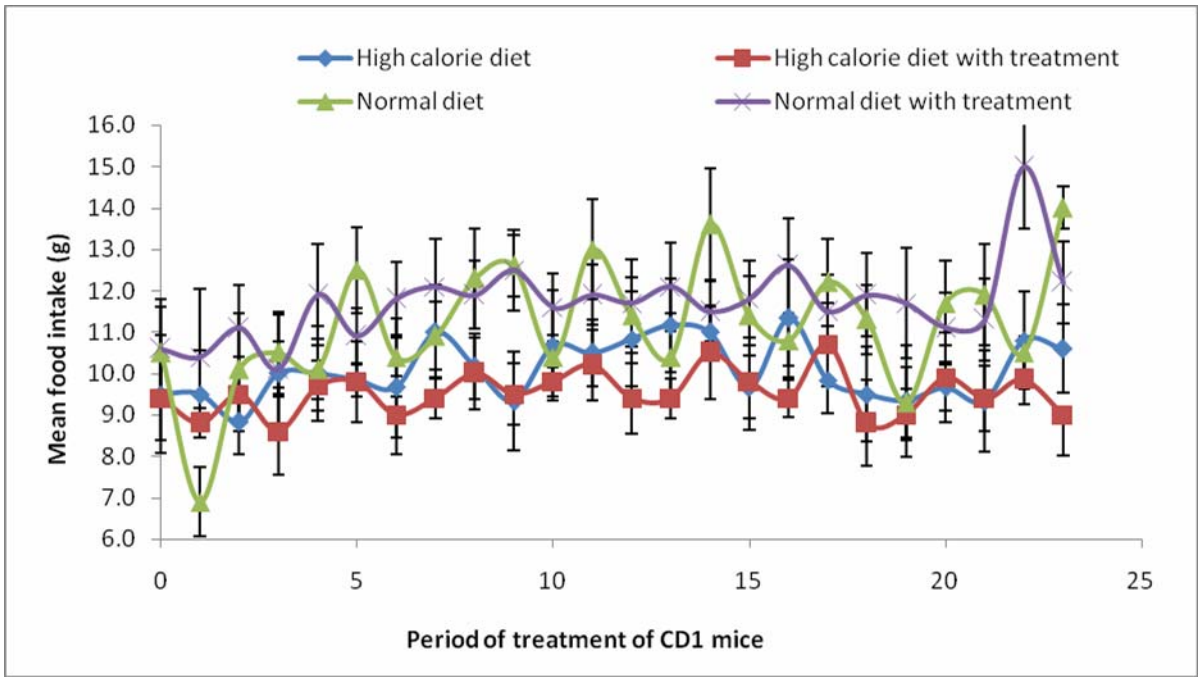


Figure 4-44The effect of high calorie and normal diet with and without treatment (the ethyl acetate fraction of *F. lutea*) on food intake of CD1 mice ((mean ± S.E.M.). Food intake at period 0 was when obesity state was attained by mice prior to commencement of treatment for about 7 weeks.

4.4.3 The effect of different diets on faecal weight

The faecal output based on weight is presented in Figure 4-45. The initial faecal weight prior to commencement of treatment was 2.00 ± 0.36 g (high calorie diet group), 2.10 ± 0.28 g (high calorie with treatment group), 1.80 ± 0.20 g (normal diet group) and 2.30 ± 0.26 g (normal diet with treatment group). Mice were fed their respective diet for about 7 weeks and the result of faecal weight (Figure 4-45) showed that the faecal weight for the mice on normal diet was higher than for those on high calorie diet.

A closer look also showed that mice on diet with treatment had higher faecal output than those on diet without treatment. At the end of the study the final faecal output for the mice were 2.80 ± 1.14 g (high calorie group), 1.67 ± 0.33 g (high calorie with treatment group), 6.25 ± 0.30 g (normal diet group) and 4.60 ± 0.28 (normal diet with treatment group). The high calorie diet group was significantly different to both groups on the normal diet (p□0.01) and tended towards significance against the non-treated high caloric diet group (p = 0.075).

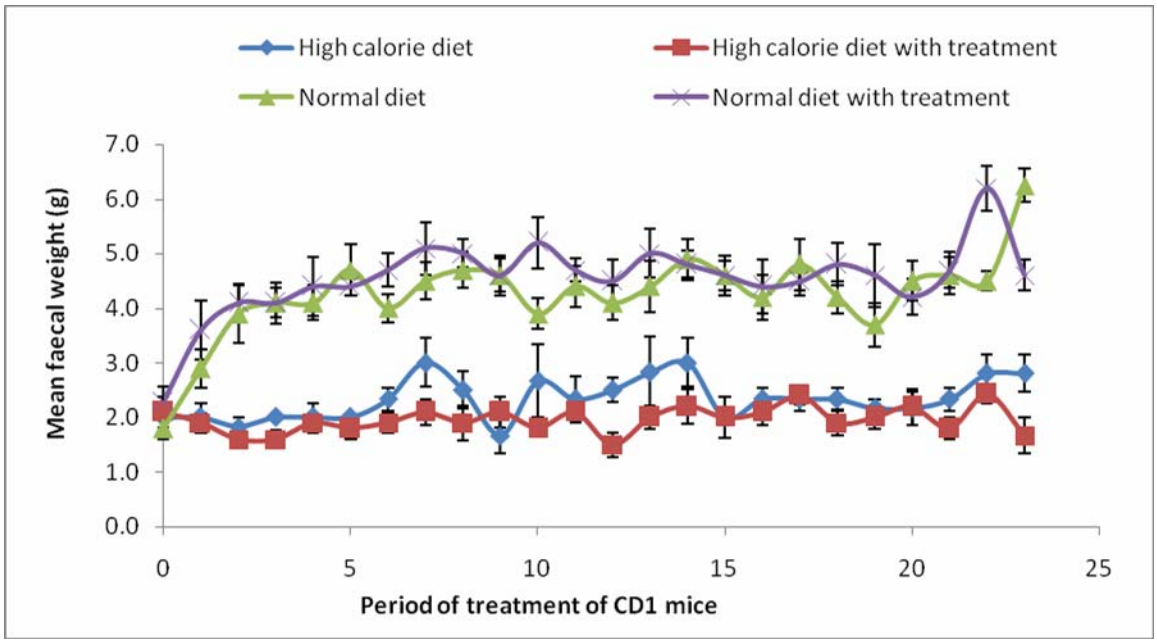


Figure 4-45The effect of high calorie and normal diet with and without treatment (the ethyl acetate fraction of *F. lutea*) on faecal output ((mean ± S.E.M.). Faecal output at period 0 was when obesity state was attained by mice prior to commencement of treatment for about 7 weeks.

4.4.4 The effect of high calorie diet on blood glucose concentration

The fasting blood glucose and glucose tolerance tests were performed on all the obese CD1 mice prior to commencement of treatment (Figure 4-46). The average fasting blood glucose concentration was 8.43 ± 1.16, 8.83 ± 0.57, 8.90 ± 0.88 and 8.82 ± 0.58 mM respectively for obese mice placed in high calorie diet, high calorie diet with treatment, normal diet and normal diet with treatment groups. There was no significant difference (p□0.05) among the treatment groups in the fasting blood glucose concentrations prior to treatment. GTT were performed on all the mice after ip injection of glucose (2 g/kg) and the average blood glucose concentrations at 5 min was 16.15 ± 4.26, 11.85 ± 1.86, 13.00 ± 2.12 and 15.68 ± 2.22 mM respectively for mice placed in high calorie diet, high calorie diet with treatment, normal diet and normal diet with treatment groups. The CD1 mice placed in the high calorie with treatment group had the lowest GTT value followed by those in the normal diet group but there was no significant difference (p□0.05) among them, those mice placed in normal diet and treatment group had the highest GTT values, followed by those in normal diet group (Figure 4-46). The blood glucose concentrations of all the mice except mice place in high calorie with treatment group continued to rise until after 90 min (Figure 4-46) when it declined to 20.92 ± 3.57, 24.26 ± 2.33 and 24.99 ± 2.09 mM respectively for mice place in high calorie diet, normal diet and normal diet with treatment groups while mice place in high calorie diet with treatment group had a decline in blood glucose concentration (18.69 ± 3.89 mM) after 30 min of glucose injection which went up again at 90

min (22.29 ± 3.82 mM). There was no significantly difference (p□0.05) in the blood glucose concentrations were present between the treatment groups.

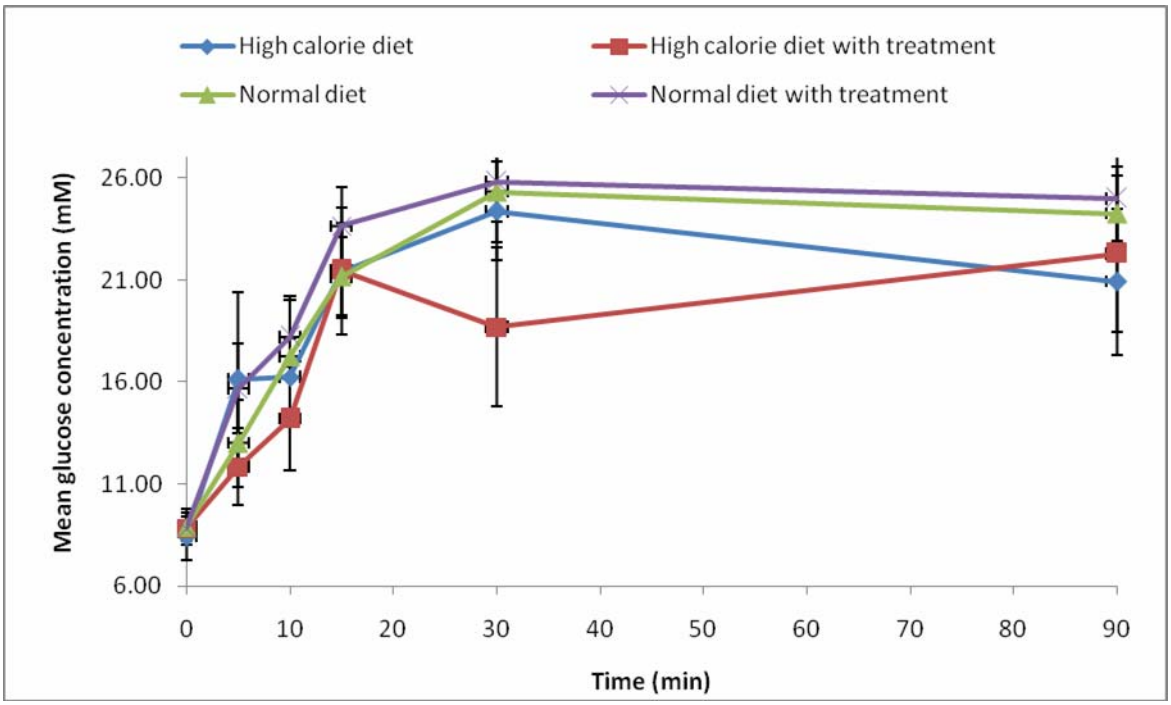


Figure 4-46The effect of high calorie diet on blood glucose concentrations of CD1 mice ((mean ± S.E.M.). Fasting blood glucose concentrations and glucose tolerance tests (GTT) at period 0 when obesity state was attained by CD1 mice prior to commencement of treatment.

The blood glucose concentrations of all the CD1 mice were evaluated again after 6 weeks of treatment (Figure 4-47) by performing the fasting blood glucose test and GTT. The average fasting blood glucose concentrations for the CD1 mice was 8.72 ± 1.16, 7.71 ± 0.57, 7.05 ± 0.88 and 7.71 ± 0.58 mM respectively for the animals in high calorie diet, high calorie diet with treatment, normal diet and normal diet with treatment groups. All the mice had fasting blood glucose concentration lower than 8 mM except the mice in the high calorie treatment group, but no significant difference (p□0.05) in the fasting blood glucose concentrations was observed among them. GTT were performed on all the mice after ip injection of glucose (2 g/kg) and the average blood glucose concentrations at 5 min was 15.72 ± 4.42, 12.23 ± 1.86, 10.96 ± 2.12 and 13.95 ± 2.22 mM respectively for mice in high calorie diet, high calorie diet with treatment, normal diet and normal diet with treatment groups. The average blood glucose concentration of the mice after 90 min of glucose administration declined for all the mice 22.97 ± 3.57 mM (mice on high calorie diet), 18.04 ± 3.82 mM (mice on high calorie with treatment), 13.22 ± 2.33 mM (mice on normal diet) and 13.38 ± 2.09 mM (mice on normal diet with treatment), with mice on normal diet and normal diet and treatment having the lowest GTT values13.22 ± 2.33 mM and 13.38 ± 2.09 mM respectively.

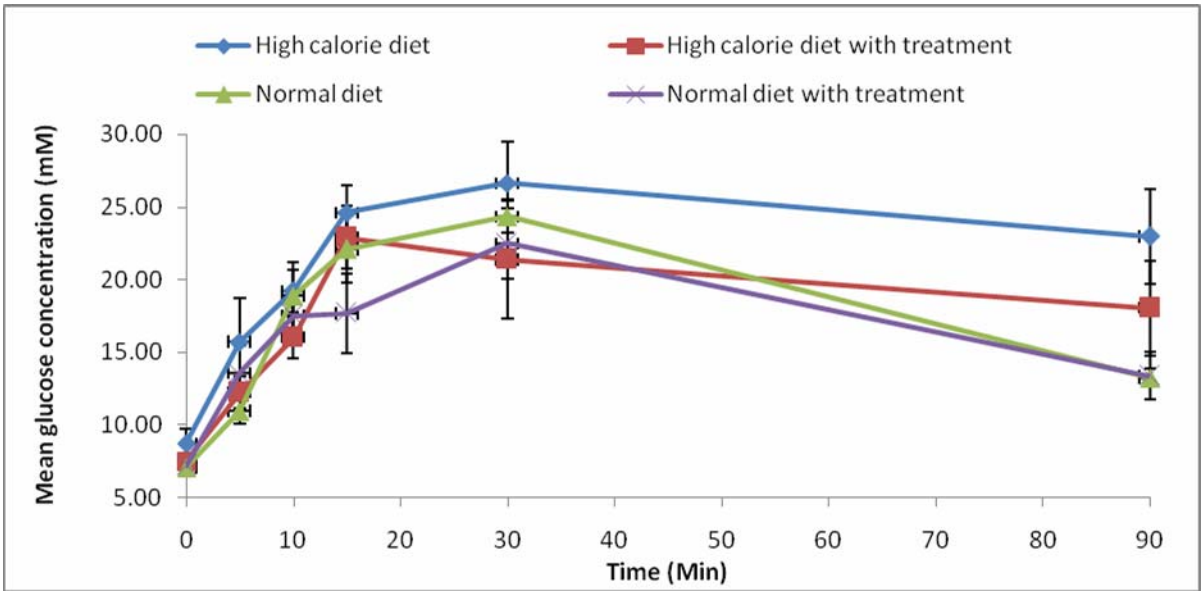


Figure 4-47The effect of different diets on blood glucose concentrations of CD 1 mice ((mean ± S.E.M.). Fasting blood glucose concentrations and glucose tolerance tests (GTT) of CD1 mice after 6 weeks of treatment.

4.4.5 The effect of different diets on full blood count parameters

The result of the haematological parameters (full blood count) is presented in Table 4-9 with no significant difference (p□0.05) between treatment groups. It was observed that the mice fed diets with treatment had lower haemoglobin, platelet and lymphocytes values than those of their respective controls. Also observed was that the RDW, WCC and neutrophils of the mice fed diet with treatment had higher values than those fed diet without treatment (Table 4-9).

4.4.6 The effect of different diets on serum chemistry parameters

The result of the serum chemistry parameters is presented in Table 4-10 with no significant difference (p□0.05) between them. All values are with the normal range for the CD1 mice. Even though values are within the normal range the alkaline phosphatase activity (ALP) and alanine aminotransferase (ALT) respectively for CD1 mice on high calorie diet with treatment and on high calorie diet. In addition, the blood glucose concentrations for the CD1 mice on diet with treatment are higher than their respective controls (Table 4-10).

4.4.7 The effect of diets on gross pathological changes in CD1 mice.

The liver, kidneys, pancreas, heart and blood vessel to the legs were collected for histological pathological examination, and there were no morphological changes observed in the kidneys, heart and blood vessel to the legs. The most consistent morphological changes in the animals were the

hypertrophic changes in the endocrine islet cells of Langerhans within the pancreas and metabolic-induce vacuolation and swelling of the hepatocytes in the liver (hepatosis). Excessive fat depots were also observed in several of the animals and excessive fat is one of the predisposing factors for diabetes. These morphological changes were induced by the high calorie diet. There was no line of demarcation between the morphological changes in mice and the diet consumed.

Table 4-9The effect of diets (with or without ethyl acetate fraction of *F. lutea*) on haematological parameters of mice

Full blood count	Group			
	High Calorie diet	High Calorie diet with Treatment	Normal diet	Normal diet with Treatment
Hb (g/l)	125.2 ± 2.94	118.6 ± 6.60	127.3 ± 3.90	124.5 ± 3.42
RCC x 10e12/l	8.6 ± 0.25	8.3 ± 0.26	8.7 ± 0.17	8.6 ± 0.14
Ht (l/l)	0.4 ± 0.01	0.38 ± 0.02	0.4 ± 0.01	0.4 ± 0.11
MCV (fl)	46.4 ± 0.52	46.4 ± 1.08	46.9 ± 0.51	46.7 ± 1.14
MCH (g/dl RC)	14.6 ± 0.17	14.2 ± 0.50	14.7 ± 0.19	14.8 ± 0.29
MCHC (g/dl RC)	31.5 ± 0.26	30.6 ± 0.46	31.3 ± 0.15	31.7 ± 0.50
RDW (%)	13.3 ± 0.44	14.7 ± 0.57	13.9 ± 0.30	15.2 ± 0.36
WCC (10e9/l)	2.5 ± 0.56	3.0 ± 0.33	1.9 ± 0.42	3.8 ± 0.65
Neuts (mat) %	21.7 ± 3.12	30.8 ± 3.91	19.4 ± 1.87	23.8 ± 3.18
N (mat) abs x 10e9/l	0.5 ± 0.12	0.9 ± 0.11	0.4 ± 0.13	0.8 ± 0.15
N (immat) %	0	0	0	0
N (immat) abs x 10e9/l	0.00	0.00	0.00	0.01 0.01
Lymph %	72.8 ± 2.20	58.7 ± 5.07	70.3 ± 3.31	68.0 ± 2.81
Lymph abs x 10e9/l	1.8 ± 0.43	1.8 ± 0.26	1.3 ± 0.22	2.3 ± 0.37
Mono %	4.2 ± 0.83	7.9 ± 1.95	6.0 ± 1.68	4.5 ± 0.81
Mono abs x 10e9/l	0.09 ± 0.02	0.2 ± 0.05	0.2 ± 0.08	0.14 ± 0.03
Eos %	1.3 ± 0.67	2.8 ± 0.91	2.9 ± 0.88	2.3 ± 0.69
Eos abs x 10e9/l	0.03 ± 0.01	0.08 ± 0.03	0.05 ± 0.16	0.07 ± 0.02
Baso %	0	0.1 ± 0.11	1.4 ±1.4	0.09 ± 0.09
Baso abs x 10e9/l	0.00	0.00	0.02 ± 0.02	0.003 ± 0.003
Plt C x 10e9/l	1133.8 ± 118.30	1037.8 ± 121.23	1231.0 ± 54.81	1174.3 ± 80.77
MPV (fl)	6.5 ± 0.24	6.5 ± 0.17	6.7 ± 0.15	6.1 ± 0.28

Table 4-10The effect of diets (with or without ethyl acetate fraction of *F. lutea*) on serum chemistry parameters of mice

Serum chemistry parameters	Group			
	High Calorie	High Calorie with Treatment	Normal diet	Normal diet with Treatment
Total protein (g/l)	51.3 ± 1.52	52.1 ± 0.90	52.2 ± 0.92	50.1 ± 0.94
Albumin (g/l)	25.2 ± 1.85	23.6 ± 1.56	24.6 ± 1.97	23.4 ± 1.06
Globulin (g/l)	26.2 ± 1.25	28.7 ± 1.00	27.9 ± 1.55	26.7 ± 1.02
Albumin/globulin ratio	1.0 ± 0.0	0.9 ± 0.11	0.9 ± 0.10	1.0 ± 0.0
ALT (U/l)	21.2 ± 1.33	150.2 ± 122.62	79.0 ± 30.50	76.6 ± 28.10
ALP (U/l)	128.7 ± 30.51	57.7± 14.44	54.5 ± 4.83	52.3 ± 3.57
Glucose (mmol/l)	9.0 ± 1.07	11.3 ± 0.91	9.3 ± 0.56	10.0 ± 0.62
Cholesterol (mmol/l)	4.7 ± 0.56	4.8 ± 0.36	3.9 ± 0.23	3.7 ± 0.24
Urea (mmol/l)	4.5 ± 0.34	4.1 ± 0.42	9.3 ± 0.50	8.4 ± 0.36
Creatinine (mmol/l)	9.0 ± 0.0	10.1 ± 1.11	9.0 ± 0.0	8.7 ± 0.27
Triglycerides (mmol/l)	1.3 ± 0.33	1.0 ± 0.17	1.3 ± 0.21	1.7 ± 0.20

Chapter 5

5 Discussion

5.1 Selection of solvent for extraction of plants

Ten *Ficus* species were selected based on availability and accessibility and were each extracted with acetone, chloroform and hexane.This did differ from traditional practice as health practitioners would prepare herbal remedies with either water or alcohol (Kelmanson*et al.*, 2000). Unfortunately these solvents extract mainly polar compounds thus limiting the amount of extractable active compounds derivable form plants. Additionally polar plants extracts are known to contain glucose and other monosaccharide sugar, which if present would complicate carbohydrate hydrolysing enzyme inhibition assays (Ali *et al.*, 2006). The presence of sugars can interfere with bioassays that measure glucose concentrations. For instance, the assay for α-amylase enzyme activity involves incubating the enzyme with standard concentration of starch as substrate, resulting in the release of the reducing disaccharide maltose (Bernfeld, 1955). In the presence of the α-amylase inhibitor, the amount of maltose released will be reduced and quantified as a percentage of the starting concentration. However, if exogenous carbohydrates are present, this can result in an inaccurate result as the starting concentration of starch could be increased to an unknown level.

Acetone was also specifically included as it is an intermediately polar solvent extractant. Martini and Eloff (1998) showed in their study that the polarity of an extracting solvent plays an important role in the quantity of mass extracted. The authors said that the polar and intermediate polar solvents have higher extracting abilities than the non-polar solvents. They said further that acetone always extracted a greater variety of compounds and the larger the variety of compounds that can be extracted by solvents, the better the chance that biologically active components will also be extracted (Eloff, 1998a).

5.2 Efficacy of crude extracts of the ten *Ficus* species

The most challenging goal in the management of diabetes mellitus is to achieve blood glucose levels as close to normal as possible (Tiwari and Rao, 2002). Postprandial hyperglycaemia is the earliest metabolic abnormality to occur in Type II diabetes mellitus (Lebovitz, 1998). Postprandial blood glucose levels may be elevated while fasting plasma glucose is normal, constituting an early stage of Type II diabetes referred to as postprandial diabetes (Baron, 1998). Alpha-amylases are endoglucanases, which hydrolyse the internal α-1,4 glucosidic linkages in starch, and α-glucosidase (sucrase), one of the glucosidases located in the brush border surface membrane of intestinal cells, is a key enzyme for carbohydrate digestion and absorption. These enzymes have been recognized as therapeutic targets for modulation of postprandial hyperglycaemia. It has also been speculated that these enzymes could be target site for the selected plants as they are all used by the oral route in

traditionally. For this study the inhibitory effectiveness of the acetone extract of the leaves of ten *Ficus* species against α-amylase and α-glucosidase were investigated and their possible relationship with total polyphenolic content and antioxidant activity was also studied. This is because the polyphenolic extracts from a number of plants have been known to be effective inhibitors of intestinal α-glucosidase and α-amylase enzymes, demonstrating their potential therapeutic effect on post-meal blood glucose levels (McDougall *et al.*, 2005).

The ten *Ficus* species were generally potent inhibitors of activity of the porcine pancreatic α-amylase but weaker inhibitors of activity of the rat small intestinal α-glucosidase. The extract of *F. sycomorus* inhibited the activity of α-glucosidase with the lowest EC_{50} of 217 ± 69 µg/ml albeit non-significantly to the EC_{50} of *F. lutea*. Some authors have shown that the extracts of other *Ficus* species such as *F. racemose* (Ahmed and Urooj, 2010), *F. benghalensis* (Ahmed *et al.*, 2011) and *F. deltoidea* (Farsi *et al.*, 2011) inhibited the activity of sucrase with an EC_{50} of 367 ± 15.2 µg/ml and 239 ± 14.3 µg/ml respectively for cold and hot water extract of *F. racemosa*, and 193 ± 21.6 µg/ml and 141 ± 22.1 µg/ml respectively for cold and hot water extract of *F. benghalensis*. This was in agreement with our results. Furthermore, studies also showed the activity of α-amylase is inhibited with an EC_{50} of 0.94 ± 0.15% and 0.58 ± 0.15% respectively for the cold and hot water extracts of *F. racemosa,* (Ahmed and Urooj, 2010) and, 4.4 and 125 µg/ml respectively for the cold and hot water extracts of *F. benghalensis* (Ponnusamy *et al.*, 2011), which was once again in agreement with our results.

While the active agent(s) responsible for the effect seen being unknown, it was speculated that the effect could be due to the presence of the polyphenolic compounds. From previous studies it was observed that the potency with which flavonoidsinhibited the activity of porcine pancreatic α-amylase and rat small intestinal α-glucosidase are different (McDougall *et al.*, 2005;Tadera *et al.*, 2006). Strawberry and raspberry extracts were the most effective inhibitors of α-amylase followed by blueberry and blackcurrant. Although these extracts also inhibited rat intestinal α-glucosidase activity, the order of effectiveness was different than forα-amylase as blueberry and blackcurrant were the most effective followed by strawberry and raspberry (McDougall *et al.*, 2005). It would therefore appear that rat intestinal α-glucosidase is generally weakly inhibited by many flavonoids, while flavonoids are often potent inhibitors of porcine pancreatic α-amylase (Tadera *et al.*, 2006). It is therefore possible that the extracts of the *Ficus* species used in this study demonstrated potent α-amylase inhibitory activity because they contain more of the flavonoid groups. To test this hypothesis, the correlation between total polyphenolic content and inhibitory of α-amylase and α-glucosidase activity was ascertained. For all ten *Ficus* species the correlation coefficient varied between 0.81 and

0.85, indicating the likelihood of the polyphenols in the acetone extracts being partly responsible for the inhibition of the activity of the enzymes.

To better evaluate study the relationship between polyphenolic content and enzyme interaction, the kinetics of inhibition for the most active extract (*F lutea*) was evaluated. With the polyphenols being present, it was speculate that the interaction with the enzymes would be non-competitive (Dixon and Webb 1999). As expected the extract of *F. lutea* showed partial non-competitive inhibition against porcine pancreatic α-amylase and α-glucosidase. Thisshowed that polyphenols (tannins) non-selectively bind to and precipitate proteins (digestive enzymes) thereby decreasing the efficiency of the enzymes.

To further establish the polyphenolic compounds as the main compounds responsible for the enzyme inhibitory effects seen, the antioxidant activity of the plant extracts was also evaluated. The extracts of *F. glumosa, F. sycomorus* and *F.lutea* both possessed good antioxidant activities with correlation with polyphenolics content being 0.62. Based on the presence of polyphenolic compounds and anti-oxidant activity, it was speculated the high molecular weight polyphenols, such as catechins, pelargonidins or leucopelargonidin may be possible active ingredients derivatives in addition to the flavonoids (Manian *et al.*, 2008). This was based on findings of Ivanova *et al.* (2005), who found that not all polyphenolic compounds possess $ABTS^{+}$ radical scavenging activities with activity being restricted to the higher molecular weight moleculesin this class (Hagerman *et al.* 1998). Another important finding of this study was the antioxidant activity seen was in agreement with other studies where the *F. glumosa*(Madubunyi *et al.*, 2012), *F. sycomorus* (Abdel-Hameed, 2009) and *F.lutea* (Marwah*et al.*, 2006) have very good DPPHscavenging activities with reported IC_{50} of 79.5 ± 1.77 µg/ml and 11.9 ± 0.3 µg/ml respectively for *F. sycomorus* and *F. lutea*. This was important as it suggests that the secondary metabolites responsible for the observed effect may be part of the natural constituent of the plant and not a stress produced mediator.

In the *in vitro* glucose uptake assays in primary cell cultures, only the extracts of *F. lutea* significantly increased in a dose related manner glucose uptake into primary muscle cell cultures, while the extracts of *F. lutea* and *F. glumosa* significantly increased in a dose related manner glucose uptake into primary fat cell culture. The extract of *F. lutea* enhanced glucose uptake into primary muscle and fat cell cultures at basal glucose concentration i.e. in the absence of insulin possible through the involvement of residual insulin receptor binding within the primary cultures preparation (Gray *et al.*, 2000), or possibly that the extract of *F. lutea* enhanced glucose uptake into the primary cultured muscle and fat cells via the same pathway as the insulin (Gray *et al.*, 2000), therefore enhancing

glucose utilisation activities in the primary cell cultures in an insulin-mimetic manner. Similarly, in the *in vitro* glucose uptake assays in the established cell lines, only the extracts of *F. lutea* significantly increased a dose related uptake of glucose into C2C12 muscle and H-4-II-E liver cells but not the 3T3-L1 pre-adipocytes in the absence of insulin. The effect was also in part enhanced by the addition of insulin to the cell wells. Based on these results the antidabetic activity of the extracts of *F. lutea* could be related to the enhancement of glucose uptake by the muscle and the liver cells.

Based on the specific effect seen in the different cell culture it is believed that the effect seen was due to an increase in the translocation of GLUT4 transported to the cell membrane. This was based on the increase in glucose uptake in the primary muscle, primary fat cell culture, C2C12 muscle and H-4-II-E liver with an absence of uptake in non-differentiated adipocytes. At the molecular level the only known differences between these cells is the absence of the GLUT4 receptor in the pre-adipocytes. Based on the speculation of activity being related to the presence of polyphenolic compounds (as discussed above), a similar effect was evident *in vitro* with plant polyphenols in muscle and liver cultures (Cazarolli *et al.*, 2008). The polyphenolic compounds are believed to enhance glucose uptake through the stimulation of the GLUT4 receptor translocation to the plasma membrane in a an insulin mimic manner by directly acting on specific components of insulin signalling transduction pathway (Pinent *et al.*, 2004) or by elevation of GLUT4 gene expression and protein levels (Liu *et al.*, 2006). For this study we believe that the former may explain the evident effect as the concurrent administration of insulin failed to significantly enhance glucose uptake. From normal physiology, the mechanism controlling the GLUT4 receptor translocation from the intra-cellular environment is mainly through the insulin receptor activity. With the number of GLUT4 receptors being fixed, it is most likely that the phenomenon of tacchyphylaxis resulted i.e. a maximum effect was based on the limited number of GLUT4 receptors present as opposed to the stimulatory potential of the extract in combination with insulin. This result was similar to those observed withresveratrol, a wine polyphenol, which when used in the absence of insulin enhanced muscular uptake of glucose but when added simultaneous to insulin led to a time dependent diminishing of glucose uptake in C1C12 muscle cells (Deug *et al.*, 2008). The authors concluded that resveratrol stimulated muscular glucose uptake via insulin independent and insulin dependent pathways in a time dependent manner (Deug *et al.*, 2008).

The extract of *F. lutea*mayalso possible also contain also glucokinin, a plant insulin-like protein. The presence of this insulin-like protein (glucokinin) has been discovered and extracted from plants with antidiabetic activity (Banting *et al.*, 1922; Xavier-Filho *et al.*, 2003). Glucokinin exhibit similar metabolic functions as insulin from animals and initiate metabolic activities in carbohydrate

metabolism (Sangeetha and Vasanthi, 2009). Plant insulin is found to be effective in regulating blood glucose by mimicking insulin signal in animals. For this insulin-like protein to be effective it has to be ingested together with protease inhibitors to protect it from hydrolysis in the digestive track (Sangeetha and Vasanthi, 2009).

The potential of the acetone extract of *F. lutea* to stimulate insulin secretion in RIN-m5F pancreatic cell line under basal condition was investigated. Results revealed that the acetone extract of *F. lutea* was capable of stimulating the pancreatic β-cells to increase the release of insulin in dose responsive manner. The extracts of *F. lutea* stimulated insulin secretion (4.58 fold) in the RIN-m5F pancreatic cells at the highest concentration in comparison to the lowest concentration while cell viability was reduced by 17.8%. The positive control, glibenclamide stimulated 1.19 fold increase insulin secretion. While our result thus suggests that the extract of *F. lutea*couldpossess insulin secretagogue properties, the effect may be due to cell membrane lysis and release of stored insulin granules. The presences of cytotoxic compounds such as glycosides in plant extracts have been demonstrated experimentally to compromise cell membranes by cell lysis which in turn could lead to the release of insulin (Persaud *et al.*, 1999). Studies have shown that the extract of *Gymnema sylvestre,* a plant that plays key role in Ayurvedic medicine stimulates insulin release *in vitro* but the mechanism is by cell membrane disruption (Persaud *et al.*, 1999).

However, despite the promising *in vitro* activity, the ability of the plant extracts to inhibit enzymes activity permanently does indicate a potential for the extract to be toxic. For this study we selected a hepatocyte and renal cell culture as these two cell types represent the most susceptible cells in the body due to their high metabolic activity, high perfusion and special transport systems that allow for the bioaccumulation of toxins. In general the ten *Ficus* species showed that the extracts were generally more toxic to the Vero kidney cells than to the C3A liver cells. However the extracts of *F. lutea* and *F. polita* were generally more toxic to the C3A liver cells than to the Vero kidney cells. Although the extracts of the *Ficus* species seemed to contain toxins that are selectively nephrotoxic, in order to avoid overestimation or underestimation of the cytotoxicity, it was suggested that more than one assay should be used to determine cell viability in *in vitro* studies, as this would increase the reliability of the results obtained (Fotakis and Timbrell, 2006). Safety of plant medicine needs to be carefully considered, investigated and validates (Halberstein, 2005), more especially when traditional use of plant extracts have been reported to cause deaths due to toxic effects of some extracts (Winslow and Kroll, 1998).

5.3 Isolation of the active components from the acetone extract of *F. lutea*

Since the extract of *F. lutea*was rich in polyphenols, possessedantioxidant activity, was a potent inhibitor of α-amylase and α-glucosidase activity, enhancedglucose uptake in cells and was a possible stimulator of insulin release, it was chosen for further studies. The active extract of *F. lutea* was fractionated using solvents of various polarities from the least non-polar (hexane) to the most to polar (water) to yield six fractions (hexane, chloroform, dichloromethane, ethyl acetate, n-butanol and water fractions). The fractions were subjected to evaluation in the α-amylase and α-glucosidase inhibition assays. These assays were selected for bio-guided fractionation as the crude extract demonstrated high inhibitory activity in previous tests (sections 4.1.5 and 4.1.6). In addition, the inhibition of these enzymes has been suggested as a means of modulating post-prandial hyperglycaemia. The n-butanol fraction was the most active in the inhibition of the α-amylase enzyme, followed by the ethyl acetate fraction and the opposite for the inhibition of α-glucosidase enzyme activity. The inhibition of α-glucosidase (sucrase) activity of the ethyl acetate and n-butanol fractions are more potent than that of the crude acetone extract of *F. lutea* with the ethyl acetate fraction being the most potent, while forα-amylase activity the n-butanol fraction was not as active as the crude extract of *F. lutea*. For the α-glucosidase (sucrase) activity, it appeared that the fractionation process resulted in potentization while for α-amylase activity was loss. The loss of activity was difficult to explain, except perhaps that the effect was non-specific and due to the additive effect of a number of different compounds of different polarity.

All the fractions of the acetone extract of *F. lutea* were generally less toxic than the crude extract except the ethyl acetate and n-butanol fractions. Although the ethyl acetate fraction was relatively less toxic, it more cytotoxic against the Vero kidney cells than the crude extract of *F. lutea* while the n-butanol fraction was more toxic against the C3A liver cells than the crude extract indicating that the toxic compounds responsible for the cytotoxicity of the extract of F. lutea against the C3A liver cells reside in the n-butanol fraction. The ethyl acetate (100.1 ± 1.5 mg/g dry weight of extract) and the n-butanol (79.58 ± 0.5 mg/g dry weight of extract)fractions of *F. lutea* contained the highest polyphenolic compounds. Sincethepolyphenols are known to interact with proteins whether dietary protein or enzymes to form complexes with and precipitate them (Gyémánt *et al.*, 2009), this characteristic of polyphenols may also account for toxic activity of the ethyl acetate and n-butanol fraction.

The ability of the fractions of acetone extract of *F. lutea* to enhance glucose uptake in C2C12 muscle cells and H-4-II-E liver cells was evaluated. The ethyl acetate and n-butanol fractions were the only fractions that significantly increased in a dose related manner glucose uptake, with the ethyl acetate

fraction being superior. When insulin and the ethyl acetate fraction were added simultaneously to the C2C12 muscle and H-4-II-E liver cells, there was neither synergistic nor additive effect rather there was an inhibition of insulin mediate glucose uptake. This result once again supported the presence of synergismof the extract.

The potential of the ethyl acetate fraction of the acetone extract of *F. lutea* to stimulate insulin secretion in RIN-m5F pancreatic cell line under basal condition was investigated. Resultsindicatethat the ethyl acetate fraction was capable of stimulating the pancreatic β-cells to increase the release of insulin in dose responsive manner. The maximum stimulation of insulin secretion got lower with potentiation. The ethyl acetate fraction stimulated insulin secretion of 3.49 fold in RIN-m5F pancreatic cells with a reduction in cell viability by about 41.7%. This result once again tends to suggests that the ethyl acetate fraction probably increasesinsulin releasethrough cell lysis.

5.4 The isolated compounds from the ethyl acetate fraction of *F. lutea*

Five polyphenolic compounds were isolated from the ethyl acetate fraction of *F. lutea*. Of the five compounds were isolated, epicatechin and epiafzelechin were isolated from this plant for the first time. All the compounds isolated except epiafzelechin have been reported in literature to have antidiabetic activity. As far as could be established, this is the first study to demonstrate that epiafzelechin has hypoglycaemic activity. In this study, epicatechin, epiafzelechin and stigmasterol in decreasing order were potent inhibitors of sucrase activity and their potency was superior to that of the crude extract of *F. lutea* as well as to the ethyl acetate fraction. Furthermore, epicatechin and epiafzelechin also enhanced superior glucose uptake in C2C12 muscle cells and H-4-II-E liver cells above those of the crude extract and the fraction and their mechanism of action was probably via the insulin-mimetic mode of action.

In the*in vitro* assay, lupeol and α-Amyrin acetate isolated from the ethyl acetate fraction of *F. lutea* did not inhibit sucrase activity nor enhance glucose uptake. Deutschlander *et al.* (2011) found that the lupeol isolated from *Euclea undulate* in *in vitro* assay inhibited α-glucosidase activity whereas Rahman *et al.* (2008) and Mbaze *et al.* (2007) found that the lupeol isolated from *C. intybus* and *Fagara tessmannii* respectively did not *in vitro* inhibit α-glucosidase activity. Oral administration of α-Amyrin acetate from *F. bengalensis* (Singh *et al.*, 2009) and *F. racemosa* (Narender *et al.*, 2009) was seen to lower blood glucose profile in streptozotocin-induced diabetic rat model but the mechanism of action was not provided. Oral administration of stigmasterol isolated from *B. monospera* decrease serum glucose concentration with a concomitant increase in insulin level (Panda *et al.*, 2009). Also in their study Deutschlander *et al.* (2011) reported epicatechin to be a potent *in*

*vitro*inhibitor of α-glucosidase activity as well as increasing glucose uptake in C2C12 muscle cells which was in agreement with our result.

When insulin and the epiafzelechin were added simultaneously to the C2C12 muscle and H-4-II-E liver cells, there was no change in glucose uptake of the cells compared to the compound in the absence of insulin indicating that the pathway by which epiafzelechin enhanced insulin mediated glucose uptake may be similar to insulin. These results was similar to those observed by Ueda *et al.* (2008), where epigallocatechin gallate stimulated a dose dependent increase in glucose uptake of L6 muscle cells with no synergism being present with insulin.

5.5 *In vivo* assay

Animals have long been used in *in vivo* diabetic experiment as alternative to the use of humans. Numerous methods exist for inducing hyperglycaemia and the diabetogenic agents often used are grouped into different classes including chemical agents, biological agents, peptides, potentiators, steroids and high calorie diet (Matteucci and Giampietro, 2008). In this study inducing obesity and its associated conditions such as insulin resistance and type II diabetes was used. It was conducted in normal male CD1 mice placed on high calorie diet *ad libitum* continuously until they became either obese or were for a total period of 13 weeks. Hereafter they were placed in one of the four treatment plans. Our result showed that the feeding of animals with high calorie can lead to increase in body mass and induce obesity. This was manifested by increased body weight and elevated blood glucose levels of most of the mice suggesting they were already in pre-diabetic state when the first glucose tolerance test was conducted. The histopathology report also suggested that the animals were prediabetic as the animals had moderate to excessive fat deposit in the abdominal cavity, mild to moderate cell swelling with vacuolated changes within the cytoplasm of the hepatocytes with or with accumulation of fatty acids suggesting metabolic-induced fatty acid changes of the liver and the enlargement of the pancreatic islets of β-cell (hypertrophic changes in the islets of the β-cell). Metabolic induced cytoplasmic vacuolation of the liver has been reported by some authors (Samuthasaneeto *et al.*, 2007; Dhibi *et al.*, 2011) who found that high fat diets and obesity enhanced the mobilisation of free fatty acid from adipose tissue and transportation into the hepatocytes. This swelling of the hepatocytes may lead to expulsion of intracellular content, indicating cell necrosis (Dhibi *et al.*, 2011), or disruption of the plasma membrane resulting into the leakage of some hepatic enzyme such as ALP and ALT into the extracellular fluid where they can be detected at abnormal levels in the serum (Dashti *et al.*, 2002). This was observed in our study asthe mice on high calorie diet and high calorie diet with treatment groups had high ALP and ALT values respectively compared to those in other groups indicative of liver damage. Hypertrophy changes in islet of β-cell have been

associated with obesity (Nugent *et al.*, 2012). The islet of β-cell being an important controlling unit of metabolism is dynamic and reactive to changes in secretory demand. Metabolic changes due to insulin insensitivity and loss of glucose control are visible by changes in islet structure.

Studies have found that feeding of CD1 mice with high calorie diet for a period of about 12 week promotes about 80% body weight gain relative to the baseline weigh (Breslin *et al.*, 2010; Hou *et al.*, 2010). The addition of the ethyl acetate fraction of *F. lutea*to the high calorie diet and normal diet of mice did not significantly reduce body weight. Hou *et al.* (2010) found a similar effect in their study in which there was lack of statistical significance between the group receiving a normal standard diet and high carbohydrate – high fat diet (HC-HF) as well as between the groups of the HC-HF diet and metformin administration. This was blamed on small number of animals in each group (n=5) as well as internal variations. FurthermoreJayaprakasam*et al.* (2006) also observed that there was no significant body weight difference between C57BL/6 mice fed high fat diet and those fed high fat diet with ursolic acid and this was similarly observed in this study. However, there was significant body weight difference between C57BL/6 mice fed high fat diet and high fat diet with anthocyanin for (Jayaprakasam *et al.*, 2006). The findings of our study are therefore difficult to explain, as our ethyl acetate fraction of *F. lutea*was rich in polyphenolic compounds. This lead to a conclusion that the sample sized used in the study may not have been sufficiently high to demonstrate such an effect.

In terms of food intake Jayaprakasam *et al.* (2006) and Tsuda *et al.* (2003) noted in their studies with C57BL/6 mice that the animals fed normal calorie diet consumed more food by weight than mice on the high fat diet with no significant difference in the caloric intake of animals. However, Hou *et al.* (2010) observed in their study with CD1 mice that the animals on high fat diet significantly consumed more food than that on normal diet and on high fat diet with metformin treatment. Jayaprakasam *et al.* (2006) observed further that the addition of anthocyanin to the high fat diet did not affect food intake nor did the addition of ursolic acid when compared to group fed high fat diet alone. This implies that anthocyanin is not an appetite suppressant as observed with the ethyl acetate fraction of *F. lutea*used in this study.

In the study by Okada *et al.* (2012) the faecal weight of rat fed normal diet with adzuki bean extracts was significantly higher than rat fed normal diet alone. Similarly the faecal weight of rat fed high calorie diet with adzuki bean extracts was significantly higher than rat fed high calorie diet alone. However, no significant difference was observed in the faecal weight of rat fed normal diet only and high fat diet alone or between those fed normal diet with treatment and high calorie with treatment. In

this study there was less faeces forthe fat diet group. This may be due to the higher nutritional value of the food, thereby results in lower waste and thus lower faecal output.

No changes in GTT were evident for this study despite positive results *in vitro*. This was an unexpected finding as, Cherian *et al.* (1992) in their study demonstrated that pelargonidin 3-O-rhamnoside isolated from the bark of *Ficus bengalensis* improved glucose tolerance in diabetic rats. This improvement in glucose tolerance was related to the compound's ability to stimulate insulin release *in vitro*. Similarly, in the study by Jayaprakasam *et al.* (2006) a mixture of pure delphinidin, cyaniding and pelargonidin 3-O-galactoside from the fruit of *Cornus mas* improved glucose tolerance in fed high fat diet and was similar to the control group fed normal standard diet. Jayaprakasam *et al.* (2006) also noted that even though ursolic acid did not significantly decrease body weight in high fat diet mice, glucose level was corrected in all the tested animals. In this study the glucose levels of all the CD1 mice after the treatment period were not significantly different and the glucose levels (Table 4-10) were within the expected values for mice of their age group (River, 1986).

The failure of an observed effect may be due to several factors. The dose is certainly a factor as the dose administer to the animals (1 mg ethyl acetate fraction/kg food) may contain very low amount of active compounds to produce physiological response. A physiological plasma concentration of not exceeding 10 µmol/l should be attained and sustained after the consumption of extract to exert biological effects (Williamson and Manach 2005). An oral dose of epigallocatechin gallate (EGCG) of 50 mg/kg body weight given to volunteers yielded a peak plasma concentration of about 0.15 µmol/l 1 h after consumption (Williamson and Manach 2005). Another contributing factor may be the mode of administration. Although the number and the concentration of the active compounds present in the ethyl acetate is unknown,direct oral administration of the fraction could have elicited better result than the addition of the fraction to diet. Oven drying the experimental food to which treatment has been added at 70^{o}C could have effect of the compounds. Nonetheless the most likely reason is the bioavailability of the active compounds. Manach *et al.* (2004) in their review noted that many excellent results in*in vitro* assays failed in *in vivo* assays mainly because many polyphenols probably have lower intrinsic activity or because they are poorly absorbed from the intestine, highly metabolised or rapidly eliminated. They said further that the metabolites that are found in the blood and target organs after digestion or hepatic modification most often differ from the native substances in terms of biological activity. It was speculated that most polyphenols are not absorbed and metabolised probably because of the hydrophilicity which restrict their ability to penetrate the gut wall by passive diffusion (Manach *et al.*, 2004). In addition because most polyphenols are in the glycosylated forms and glycosylation influences absorption, it was also speculated that most of the

glycosides resist acid hydrolysis in the stomach and thus remain in their intact form on reaching the duodenum Manach *et al.*, 2004).

Chapter 6

6 Conclusion

This study investigated the potential antidiabetic activity of ten *Ficus* species, focussing on digestive enzymes in relation to polyphenol content and antioxidant activity, glucose uptake in muscle, fat and liver; insulin secretion and safety through cytotoxicity assay. All acetone extracts of the ten *Ficus* species had α-amylase and α-glucosidase enzyme inhibitory activity. The crude acetone extract of *F. lutea* was the most active in all the assays. The acetone extract of *F. lutea* was the most effective in stimulating glucose uptake in the primary muscle and fat cell culture as well as in established cell lines of C2C12 muscle and H-4-II-E liver but not in undifferentiated 3T3-L1 pre-adipocyte in the absence and presence of insulin indicating an insulin-mimetic and insulin sensitising as a possible mode of action. The extract of *F. lutea* in addition enhanced insulin secretion in RIN-m5F pancreatic cell line. Although the extract of *F. lutea* was relatively less cytotoxic to Vero kidney cells it was more cytotoxic to C3A liver cells when compared to the other *Ficus* species.

The extract of *Ficus lutea,* being the most active, was fractioned by solvent-solvent fraction into six fractions. These fractions were subjected to the above assays and ethyl acetate fraction was the most active fraction ininhibiting α-glucosidase (sucrase) activity in a manner that was more potent than the crude, with good correlated withits polyphenol content. The ethyl acetate fraction was superior to the crude extract in enhancing glucose uptake in C2C12 muscle and H-4-II-E liver in the absence of insulin. The mechanism through which the ethyl acetate fraction enhanced glucose uptake is uncertain but it is probably similar to that for insulin. The ethyl acetate fraction was relatively more cytotoxic to Vero kidney cells than the other fractions and the crude extract.

Five compounds, namely lupeol, stigmasterol, α-amyrin acetate, epicatechin and epiafzelechin were isolated from the ethyl acetate fraction of *F lutea*, with all of the compounds except epiafzelechin previously known to possess antidiabetic activity. The ethylacetate fraction was also evaluated for its weight reducing potential in obese mouse model. Unfortunately no *in vivo* activity was discernible.

Type II diabetes affects many metabolic pathways in different tissues which are potential targets for drug treatment (Van de Venter *et al.*, 2008). Conversely, the extract of *F. lutea* consists of some phytochemicals that has been shown to possess antidiabetic activities. It can then be speculated that the mechanisms underlying the antidiabetic activity of the extract of *F. lutea* includes the inhibition of α-amylase and α-glucosidase activities (enzymes responsible for the breakdown of carbohydrate), enhancing of glucose uptake in muscle, fat and liverand by stimulating the secretion of insulin. In conclusion, this study is the first to report on the *in vitro* antidiabetic activity of the extract of *F. lutea*.

6.1 Future Work

More extensive *in vitro* cytotoxicity evaluation of the extract of *F. lutea* and *in vivo* acute and chronic toxicity assay is needed to ascertain its safety especially the target organ for toxicity.

As the n-butanol fraction of the extract of *F. lutea*inhibited α-amylase and potential to stimulate glucose uptake in muscle and liver cell lines, it may be worthwhile to investigate its stimulatory effect in insulin stimulated glucose uptake in muscle, liver and fat cells.

A repeat of the failed obesity induced diabetic animal study may reveal new finding perhaps with animals being treated with oral gavage dose of the ethyl acetate fraction of the extract of *F. lutea*. The oral dosing of animals may allow for higher concentration of the ethyl acetate fraction to be in contact with target tissues/organs.

Since Type II diabetes is a multi-faceted disease a general evaluation of the hypoglycaemic activity of the extract of *F. luteain vivo* in a natural diabetes rodent could give an indication of its potential as an antidiabetic agent.In further work the following aspects may be addressed:

- To what extent will the complications of type II diabetes be reduced by *F. lutea* extracts and do the extracts of *F. lutea* reduce the extent of oxidative stress leading to these complications?
- Does ingestion of polyphenolic compounds present in *F. lutea* influence endogenous antioxidant enzymes and non-enzymatic reactions?
- Could the measurement of glycated haemoglobin (HbA1c) in the rodent blood be a better way of evaluating the management of type II diabetes in a mouse obesity model subjected to the *F. lutea* extract?