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Original Article

The effects of *Ficus carica* on the activity of enzymes related to metabolic syndrome

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ABSTRACT

The present study aimed to investigate the effects of the various parts of *Ficus carica* L. (figs) on antioxidant, antidiabetic, and antiobesogenic effects *in vitro*. Fruit, leaves, and stembark of the *F. carica* plant were sequentially extracted using organic and inorganic solvents and their total polyphenol and flavonoid contents were estimated. The effects of the extracts on antioxidative, antidiabetic (inhibition of α-amylase and α-glucosidase enzymes), and antiobesogenic (antilipase) activities were measured using several experimental models. The fruit ethanolic extract contained a high quantity of polyphenols and flavonoids (104.67 ± 5.51 μg/mL and 81.67 ± 4.00 μg/mL) compared with all other extracts. The activity of the ethanolic extract of *F. carica* fruit was significantly ($p < 0.05$) higher than all other extracts and parts of the plant in terms of antioxidative, antidiabetic, and antiobesogenic effects. The IC_{50} values of the fruit ethanolic extract in terms of antioxidative (134.44 ± 18.43 μg/mL), and inhibition of α-glucosidase (255.57 ± 36.46 μg/mL), α-amylase (315.89 ± 3.83 μg/mL), and pancreatic lipase (230.475 ± 9.65 μg/mL) activity indicate that the activity of fruit ethanolic extract is better than all other extracts of the plant. The gas chromatography–mass spectroscopy analysis of the fruit ethanolic extract showed the presence of a number of bioactive compounds such as butyl butyrate, 5-hydroxymethyl furfural, 1-butoxy-1-isobutoxy butane, malic acid, tetradecanoic acid, phytol acetate, *trans* phytol, *n*-hexadecanoic acid, 9Z,12Z-octadecadienoic acid, stearic acid, sitosterol, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one, and 2,4,5-trimethyl-2,4-dihydro-3*H*-pyrazol-3-one. The results of this study suggest that the ethanolic extract of the fruit of *F. carica* may have potential antidiabetic and antiobesogenic agents.

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1. Introduction

Obesity and diabetes are two interrelated and world-wide global public health problems. The prevalence of both of the diseases has been increased tremendously in the past few decades. The highest rate of obesity and overweight among adults in Sub-Saharan Africa is found in South Africans (women at 42% and men at 39%), when the combined percentage of overweight and obese people is 69.3% [1]. The World Health Organization (WHO) showed that, globally, there are > 1 billion adults who are overweight and 300 million people are living with obesity [2,3]. Although the 2008 data from the WHO indicate that > 115 million people have obesity associated diseases in developed countries [2], obesity is no longer a problem of developed countries only. It is gradually becoming an increasing problem in developing nations and countries undergoing epidemiological transition, such as South Africa, Mexico, and so on [4,5]. The coexistence of obesity along with diabetes, cardiovascular diseases, cancer, and atherosclerosis could negatively affect life span and the quality of life of many people around the world, particularly for the people in obesity prevalent countries; however, the existence of diabetes is higher than other noncommunicable diseases [6].

Diabetes has been recognized as one of the closely associated problems with obesity in past decades. The major basis for this link is the obesity associated resistance of available insulin in the cells and the destruction of insulin secretory mechanisms [7]. Insulin resistance is one of the two major pathogeneses of type 2 diabetes (T2D) and some other pathophysiological conditions such as hyperlipidemia, hypertension, and hyperglycemia [7]. The high prevalence of obesity might be partly involved in the higher percentage of T2D in African population. In 2010, > 12.1 million people were estimated to have diabetes in Africa; however, by the end of 2014, the number of diabetes patients had increased to 20 million. This figure is projected to increase to 23.9 million by the end of 2030 [8,9]. The increased number of population with these two metabolic disorders (obesity and T2D) and the consequence of other communicable diseases contribute to the increasing cost of healthcare expenditures which may not be affordable by people in most developing countries including Africa [6]. Although, different approaches are being used to treat diabetes and obesity, the inhibition of carbohydrate and lipid digesting enzymes such as α-glucosidase, α-amylase, and pancreatic lipases, respectively, is one of them. The inhibition of these enzymes not only reduces the digestion and absorption of carbohydrates and lipids but also reduces the blood glucose and body fat levels significantly. However, phytochemicals have been recognized as a cost effective alternative to modern drugs for the treatment of metabolic disorders such as diabetes and obesity [10].

Medicinal plants belonging to the genus *Ficus* have been reported as better remedies for metabolic disorders, where *Ficus carica* L. has received more attention compared with other species because its fruit is usually used as an edible food. *F. carica* belongs to the Moraceae family which is popularly called "figs." Although *F. carica* fruits are normally used as food, the polysaccharides and potential polyphenols from its fruits have been used as medicine for many years [11]. Polysaccharides present in *F. carica* fruit have been reported to have antispasmodic, antitumor, anti-inflammatory, and antioxidant properties in a number of previous studies [12–15], while its phytochemical composition as well as antioxidative, antidiabetic, and antiobesogenic effects are not well understood.

F. carica fruit phytochemical such as 6-O-acyl-β-D-glucosyl-β-sitosterol was reported as a potential cytotoxic agent and the latex of fig demonstrated the inhibition of cancer cell proliferation [16,17]. Leaves of *F. carica* also showed good antioxidant, anti-inflammatory, and hepatoprotective activity [18,19]. Various extracts of *F. carica* leaves were reported to have antidiabetic, antihyperlipidemic, and antioxidative activities in rats [20–24]. A recent comprehensive review also reported the use of *F. carica* in the treatment of anemia, cancer, diabetes, liver diseases, paralysis, skin diseases, ulcers, and suggested its potential for clinical use [25]. However, the comparative effects of the various parts of *F. carica* on antioxidative, antidiabetic, and antiobesogenic effects have not been investigated so far.

Therefore, our present study investigated the antioxidative, antidiabetic, and antiobesogenic effects of the various solvent extracts of the different parts of *F. carica in vitro*. Additionally, the gas chromatography–mass spectroscopy (GC–MS) analysis of the most active extract was conducted to identify the major bioactive compounds.

2. Materials and methods

2.1. Chemicals and reagents

Porcine pancreatic lipase (L3126), α-amylase (A3176), and yeast α-glucosidase (G5003), Orlistat (O4139), *p*-nitrophenylbutyrate (pNPB) (N9876), *p*-nitrophenyl-α-D-glucopyranoside (N1377), acarbose (A8980), gallic acid (G7384), quercetin (Q4951), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH; D9132), and ascorbic acid (A5960) were purchased from Sigma-Aldrich, Germany. Starch, dinitrosalicylic acid, absolute ethanol, ethyl acetate, hexane, and dimethylsulfoxide (DMSO) were obtained from Merck Chemicals Company, Germany.

2.2. Collection of plant materials

The stembark and leaves of *F. carica* L. were collected from Seshachalam forest, Tirupati, India, whereas the fruits were purchased from a local market in Durban, South Africa. The authentication of plant materials was done by Dr K. Madhava Chetty, Sri Venkateswara University, India. Herbarium voucher specimens (voucher number: 1139) were deposited at the department of botany herbarium of the same university. All plant materials were washed, shade dried, ground to powder or paste using a kitchen or laboratory blender, and stored in airtight containers for subsequent analysis.

2.3. Preparation of extracts

A 100-g of homogeneous powder from leaf or stembark and paste from fruit of *F. carica* were separately extracted with

hexane, ethyl acetate, ethanol, and water for 48–72 hours in 500 mL of the respective solvent, filtered through Whatmann filter paper (Number 1, Sigma-Aldrich, USA), and the organic solvents were evaporated using a rotary evaporator (Buchi Rotavapor II, Buchi, Flavil, Switzerland) at 40°C under reduced pressure. The aqueous extract was dried on a water bath at 50–55°C. All extracts were transferred to micro tubes and stored at 4°C until further analysis.

2.4. Estimation of total polyphenol content

The total polyphenol content of each extract was estimated as gallic acid equivalent according to the method of McDonald et al [26] with slight modifications. Briefly, 200 μL of the extract dissolved in 10% DMSO (500 μg/mL) was incubated with 1 mL of Folin Ciocalteau reagent (diluted 10 times) and 800 μL of 0.7 M Na_2CO_3 for 30 minutes at room temperature. Then the absorbance was then measured at 765 nm on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All measurements were done in triplicate.

2.5. Estimation of total flavonoid content

The total flavonoid content of each extract was determined (as quercetin equivalent) according to the method described by Zhishen et al [27] with slight modifications. Briefly, 250 μL of the extract dissolved in 10% DMSO (500 μg/mL) was incubated with 150 μL of 5 M $NaNO_2$. After 5 minutes, 150 μL of 10% aqueous $AlCl_3$ was added to the mixture, then 1 mL of 1 M NaOH was added to the solution after a 1-minute interval. Then the absorbance was measured at 510 nm on the same spectrophotometer as mentioned above. All measurements were done in triplicate.

Table 1 – Total polyphenols and flavonoid content of the different parts of *Ficus carica*.

Sample	Total polyphenols (mg/g GAE)	Total flavonoids (mg/g QE)
Fruit		
Hexane	10.34 ± 0.57^{a}	8.46 ± 1.1^{a}
Ethyl acetate	44.67 ± 2.51^{c}	36.17 ± 2.5^{c}
Ethanol	104.67 ± 5.51^{e}	81.67 ± 4.0^{e}
Aqueous	66.6 ± 1.63^{d}	42.33 ± 2.5^{d}
Leaf		
Hexane	ND	ND
Ethyl acetate	12.22 ± 0.124^{a}	9.24 ± 0.215^{a}
Ethanol	54.14 ± 4.325^{d}	24.52 ± 1.102^{b}
Aqueous	32.45 ± 5.058^{bc}	24.05 ± 3.021^{b}
Stembark		
Hexane	ND	7.665 ± 1.025^{a}
Ethyl acetate	15.302 ± 3.202^{a}	12.025 ± 2.302^{a}
Ethanol	48.361 ± 1.253^{c}	26.325 ± 2.402^{b}
Aqueous	28.605 ± 2.035^{b}	19.256 ± 1.115^{b}

Data are presented as mean ± SD of triplicate determinations.
$^{a-e}$ Different superscript letters within a column for a given parameter are significantly different from each other (Tukey's-honest significant difference multiple range *post hoc* test $p < 0.05$ IBM SPSS version 23).
GAE = gallic acid equivalent; QE = quercetin equivalent; ND = not determined.

2.6. In vitro antioxidant or free radical inhibition activity

The total free radical inhibition activity of the extracts was determined using a slightly modified method of Tuba and Gulcin [28], when ascorbic acid was used as a standard. Briefly, a 0.3 mM solution of DPPH was prepared in methanol and 500 μL of this solution was added to 1 mL of the extract (dissolved in 10% DMSO) at different concentrations (100–500 μg/mL). These solutions were mixed and incubated in the dark for 30 minutes at room temperature. Then the absorbance was measured at 517 nm against a blank lacking scavenger.

The antioxidant or free radical inhibition activity was calculated according to the following formula:

$$\% \text{ Inhibition} = [(AB - AA)/AB] \times 100,$$

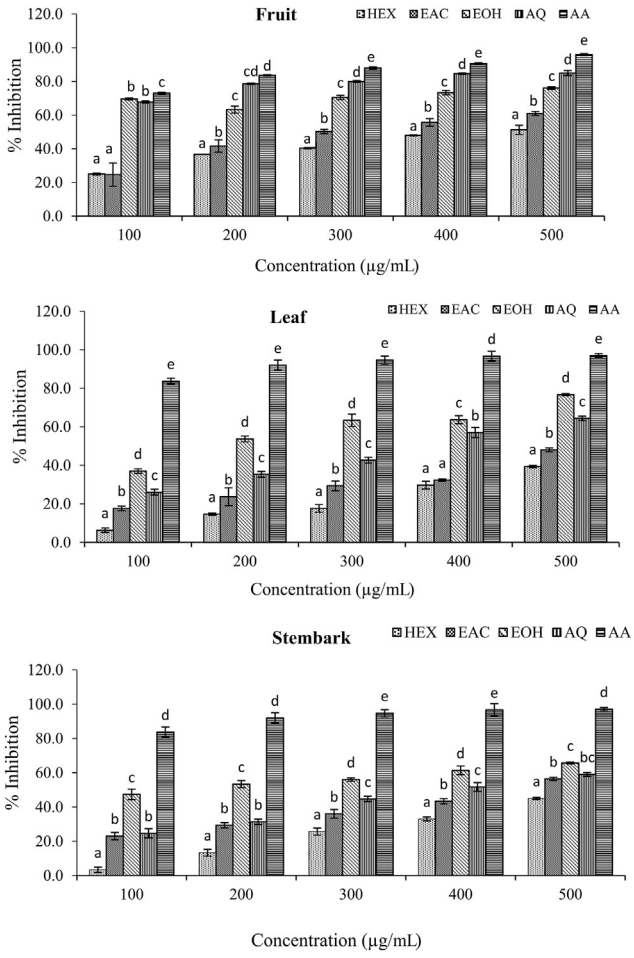

Figure 1 – *In vitro* antioxidant activity of the fruit leaves and stembark of *Ficus carica* L. Data are presented as mean ± SD of triplicate determinations. $^{a-e}$ Different letters above the bars for a given concentration are significantly different from each other (Tukey's-honest significant difference multiple range *post hoc* test $p < 0.05$ IBM SPSS version 23). AA = ascorbic acid; AQ = aqueous; EAC = ethyl acetate; EOH = ethanol; HEX = hexane; SD = standard deviation.

where AB = absorbance of the blank DPPH solution and AA = absorbance of the tested extract.

2.7. *In vitro α-glucosidase assay*

The α-glucosidase inhibitory activity of extracts was determined according to the method described by Ademiluyi and Oboh [29] with slight modifications. Briefly, 250 μL of each extract or acarbose at different concentrations (100–500 μg/mL) was incubated with 500 μL of 1.0 U/mL α-glucosidase solution in 100 mM phosphate buffer (pH 6.8) at 37°C for 15 minutes. Thereafter, 250 μL of *p*-nitrophenyl D-glucoside solution (5 mM) in 100 mM phosphate buffer (pH 6.8) was added and the mixture was further incubated at 37°C for 20 minutes. The absorbance of the released *p*-nitrophenol was measured at 405 nm and the inhibitory activity was expressed as a percentage of the control without inhibitor. All assays were carried out in triplicate and the calculation was done according to the following formula.

$$\%\ Inhibition = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.8. *In vitro α-amylase assay*

The α-amylase inhibitory activity of extracts was determined according to the method described by Shai et al [30]. A 250-μL of each extract dissolved in 10% DMSO or acarbose at different concentrations (100–500 μg mL^{-1}) was incubated with 500 μL of porcine pancreatic amylase (2 U mL^{-1}) in 100 mM phosphate buffer (pH 6.8) at 37°C for 20 minutes. A 250-μL of 1% starch dissolved in 100 mM phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 37°C for 1 hour. 1-mL of dinitrosalicylic acid colour reagent was then added and boiled for 10 minutes. Then the absorbance of the resulting mixture was measured at 540 nm and the inhibitory activity was expressed as a percentage of the control without inhibitor. All assays were carried out in triplicate and the calculation was done according to the following formula.

$$\%\ Inhibition = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.9. *In vitro pancreatic lipase assay*

The method for measuring pancreatic lipase activity was modified from that of Kim et al [31]. Briefly, an enzyme buffer was prepared by the addition of a solution of porcine pancreatic lipase (2.5 mg/mL in 10 mM morpholinepropanesulphonic acid and 1 mM EDTA, pH 6.8). Thereafter 169 μL of Tris buffer (100 mM Tris-HC1 and 5 mM $CaCl_2$, pH 7.0) was added to 100 μL of the extract (100–500 μg/mL) at the test concentration, or orlistat, after which 20 μL of the enzyme buffer was added and incubated for 15 minutes at 37°C. A volume of 5 μL of the substrate solution (10 mM *p*-nitrophenyl butyrate in dimethyl formamide) was then added and incubated for 30 minutes at 37°C. The lipase activity was determined by measuring the hydrolysis of *p*-nitrophenyl butyrate to *p*-nitrophenol at 405 nm. All assays were carried out in triplicate and the calculation was done according to the following formula.

$$\%\ Inhibition = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Table 2 – IC_{50} values of various parts of *Ficus carica* L. on *in vitro* antioxidant, α-amylase, α-glucosidase and pancreatic lipase activities.

Sample	DPPH	α-Amylase	α-Glucosidase	Pancreatic lipase
	IC_{50} (μg/mL)			
Fruit				
Hexane	1200 ± 23.37^{e}	19582.4 ± 115.60^{f}	11314.25 ± 16.8^{g}	26885.5 ± 550.9^{f}
Ethyl acetate	$770 \pm 11.35^{c,d}$	10789.69 ± 208.65^{e}	3882 ± 140.076^{f}	2492.36 ± 234.77^{e}
Ethanol	134.44 ± 18.43^{b}	315.89 ± 3.83^{a}	255.57 ± 36.46^{b}	230.475 ± 9.6521^{b}
Aqueous	162.10 ± 10.64^{b}	$369.84 \pm 3.10^{a,b}$	$320.9 \pm 51.389^{b,c}$	$310.16 \pm 13.216^{b,c}$
Leaf				
Hexane	1233 ± 40.764^{e}	11611.26 ± 318.9^{e}	1746.8 ± 401.95^{f}	113019.5 ± 317.3^{g}
Ethyl acetate	899.55 ± 109.737^{d}	2665.57 ± 514.923^{c}	1190.79 ± 84.88^{e}	2943.07 ± 220.90^{e}
Ethanol	175.857 ± 8.932^{b}	550.963 ± 33.931^{b}	271.59 ± 13.457^{b}	$364.96 \pm 2.176^{b,c}$
Aqueous	322.110 ± 12.970^{c}	687.496 ± 41.987^{b}	525.877 ± 44.23^{c}	442.8 ± 129.14^{c}
Stembark				
Hexane	736.395 ± 37.441^{d}	9998.256 ± 30.165^{e}	43131 ± 116.93^{f}	276085.1 ± 1403.4^{h}
Ethyl acetate	507.584 ± 55.794^{d}	2031.034 ± 23.540^{c}	1190.79 ± 84.88^{e}	41574.56 ± 20.25^{f}
Ethanol	171.479 ± 19.354^{b}	642.054 ± 54.032^{b}	441.08 ± 25.404^{c}	$688.56 \pm 69.9^{c,d}$
Aqueous	376.055 ± 33.931^{c}	638.601 ± 32.086^{b}	710.974 ± 31.32^{d}	842.70 ± 151.34^{d}
Ascorbic acid	1.652 ± 0.479^{a}	—	—	—
Acarbose	—	273.665 ± 27.083^{a}	82.09 ± 17.099^{a}	—
Orlistat	—	—	—	1.894 ± 0.017^{a}

Data are presented as mean ± SD of triplicate determinations.
[a–h] Different letters within a column for a given parameter are significantly different from each other (Tukey's-honest significant difference multiple range *post hoc* test $p < 0.05$ IBM SPSS version 23).
DPPH = 1,1-diphenyl-2-picrylhydrazyl radical; SD = standard deviation.

2.10. GC–MS analysis of the extracts

The most active ethanolic extract was analyzed using GC–MS. The analysis was conducted with an Agilent Technologies 6890 GC coupled with an Agilent 5973 mass selective detector driven by Agilent Chemstation software (Agilent Technologies, Santa Clara, California, USA). A DB-5SIL MS capillary column was used (30 m × 0.25 mm internal diameter, × 0.25 μm film thickness). The ultrapure helium was used as a carrier gas at a flow rate of 0.7 mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250°C. The initial oven temperature was 60°C, which was ramped up to 280°C at a rate of 10°C/min with a hold time of 3 minutes. Injections of 2-μL sample were made in the splitless mode. The mass spectrometer was operated in the electron ionization mode at 70 eV with the electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadrupole temperature 150°C, solvent delay 4 minutes, and scan range 50–700 amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology library.

2.11. Statistical analysis

Data were analyzed using a statistical software package (SPSS for Windows, version 23, IBM Corporation, New York, USA) using Tukey's-honest significant difference multiple range *post hoc* test. Values were considered significantly different at $p < 0.05$.

3. Results

3.1. Total polyphenols and flavonoids content

The results of total polyphenols and flavonoids content in the different extracts of fruit, leaves, and stembark are presented in Table 1. Among the different extracts of *F. carica*, the fruit ethanolic extract showed highest yield of polyphenols and flavonoids (104.67 mg/g and 81.67 mg/g, respectively) compared with all other extracts.

3.2. In vitro antioxidant or free radical inhibition activity

The results of *in vitro* antioxidant or free radical inhibition activity of different extracts are presented in Figure 1. Free radical scavenging activity was tested in all extracts of *F. carica*. Compared with the leaf and stembark extracts, significantly higher ($p < 0.05$) antioxidant activity was observed in the fruit ethanolic extract (Figure 1). The IC_{50} value of the fruit ethanolic extract was low (134 μg/mL) in comparison to other extracts (Table 2). The ethanolic extract of the leaf (175 μg/mL) and stembark (171 μg/mL) also showed relatively higher IC_{50} values in comparison to the ethanolic extract of fruit when the values were not significantly different.

3.3. In vitro α-amylase activity

The results of α-amylase activity are presented in Figure 2. Among all extracts of the fruit, the ethanolic extract showed the best inhibition. The leaf and stembark extracts showed moderate inhibition (Figure 2). The ethanolic extract of the fruit showed the lowest IC_{50} value (315.89 μg/mL), which is significantly ($p < 0.05$) lower than the all other extracts from leaf and stembark (Table 2).

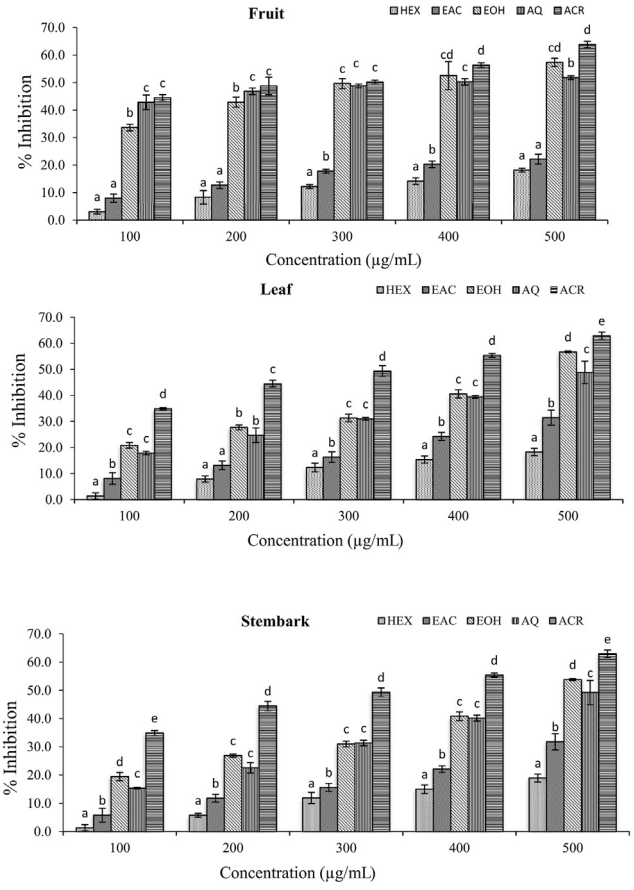

Figure 2 – *In vitro* α-amylase activity of the fruit, leaf and stembark of *Ficus carica* L. Data are presented as mean ± SD values of triplicate determinations. [a, b] Different letters above the bars for a given concentration are significantly different from each other (Tukey's-honest significant difference multiple range *post hoc* test $p < 0.05$ IBM SPSS version 23). ACR = acarbose; AQ = aqueous; EAC = ethyl acetate; EOH = ethanol; HEX = hexane; SD = standard deviation.

3.4. In vitro α-glucosidase activity

The results of the inhibitory activity of *F. carica* extracts against α-glucosidase enzyme are presented in Figure 3. Among the different extracts of the leaf, stembark, and fruit, the fruit ethanolic extract was found to be a potential inhibitor against α-glucosidase compared with all other extracts (Figure 3). The IC_{50} value of fruit ethanolic extract was also significantly ($p < 0.05$) lower when compared with other extracts (Table 2).

3.5. In vitro pancreatic lipase activity

The results of pancreatic lipase activity are presented in Figure 4. The ethanolic extract of the fruits of *F. carica* showed

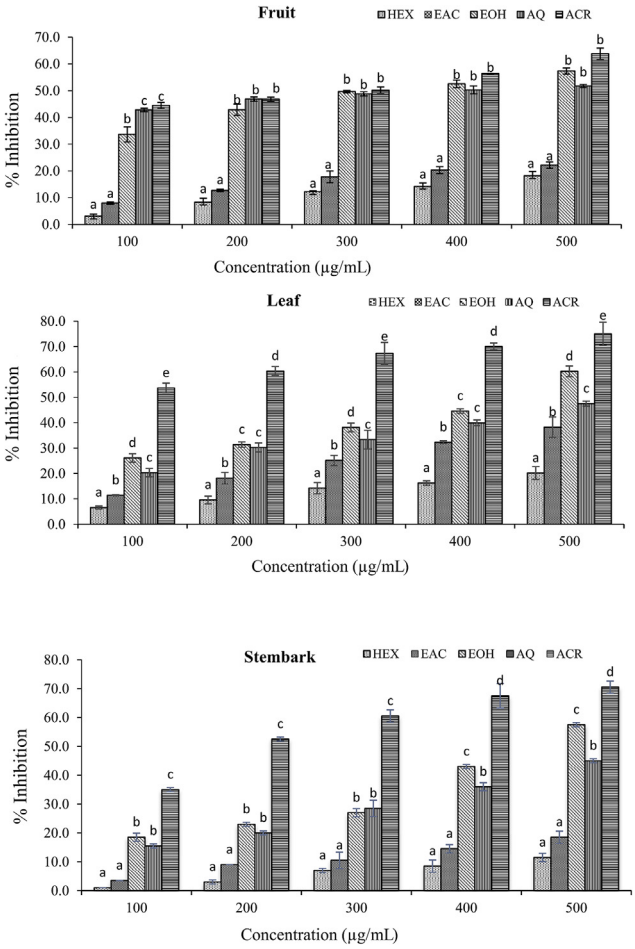

Figure 3 – *In vitro* α-glucosidase activity of the fruit leaves and stembark of *Ficus carica* L. Data are presented as mean ± SD values of triplicate determinations. [a–e] Different letters over the bars for a given concentration are significantly different from each other (Tukey's-honest significant difference multiple range *post hoc* test $p < 0.05$ IBM SPSS version 23). ACR = acarbose; AQ = aqueous; EAC = ethyl acetate; EOH = ethanol; HEX = hexane; SD = standard deviation.

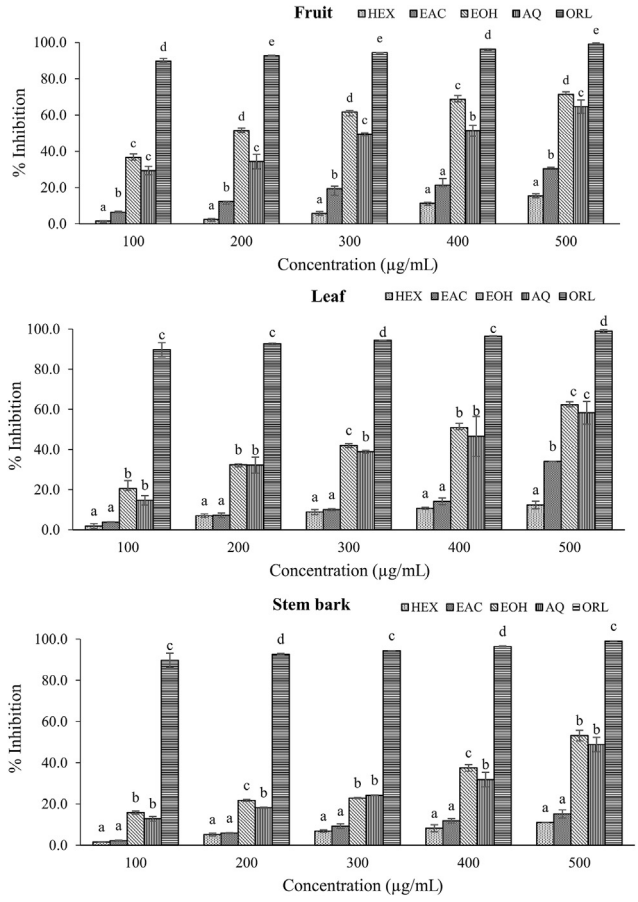

Figure 4 – *In vitro* pancreatic lipase activity of the fruit leaves and stembark of *Ficus carica* L. Data are presented as mean ± SD of triplicate determinations. [a–d] Different letters over the bars for a given concentration are significantly different from each other (Tukey's-honest significant difference multiple range *post hoc* test $p < 0.05$ IBM SPSS version 23). AQ = aqueous; EAC = ethyl acetate; EOH = ethanol; HEX = hexane; SD = standard deviation.

significant ($p < 0.05$) inhibition against pancreatic lipase compared with all other extracts (Figure 4). In addition, the IC_{50} value of fruit ethanolic extract was also significantly lower than all other extracts (Table 2).

3.6. GC–MS analysis of the fruit ethanolic extract of F. carica

GC–MS analysis of the fruit ethanolic extract of *F. carica* indicated the presence of 13 phytocompounds **1**–**13** (Figure 5), consisting of saturated organic acids (tetradecanoic acid **5**, n-hexadecanoic acid **8**, and stearic acid **10**), as well as an unsaturated organic acid, 9Z,12Z-octadecadienoic acid **9**, and a common precursor to the secondary metabolites, malic acid **4**. The esters, butyl butyrate **1**, and phytol acetate **6** were also identified in the extract. Along with phytol acetate, *trans* phytol **7** was identified. A furfural **2**, an isobutoxy ether **3**, and the ubiquitous sitosterol **11** were the other three compounds identified in the extract. The ethanolic extract contained more furfural **2**, a substituted pyrazolone **12**, and a substituted dihydropyranone **13**. The retention times, molecular ion peaks, or other prominent peaks (where molecular ion peaks were absent) and percentage area of peaks are presented in Table 3 and the structures of the compounds shown in Figure 5.

4. Discussion

In the present study, *F. carica* L. leaf, stembark, and fruit extracts were subjected to estimation of polyphenols and flavonoids content as well as DPPH radical scavenging activity. The ethanolic extracts of the fruit, which contained a high amount of polyphenols and flavonoids, showed significantly higher *in vitro* antioxidant activity compared with other solvent extracts from fruits and the all extracts from leaves and stembark

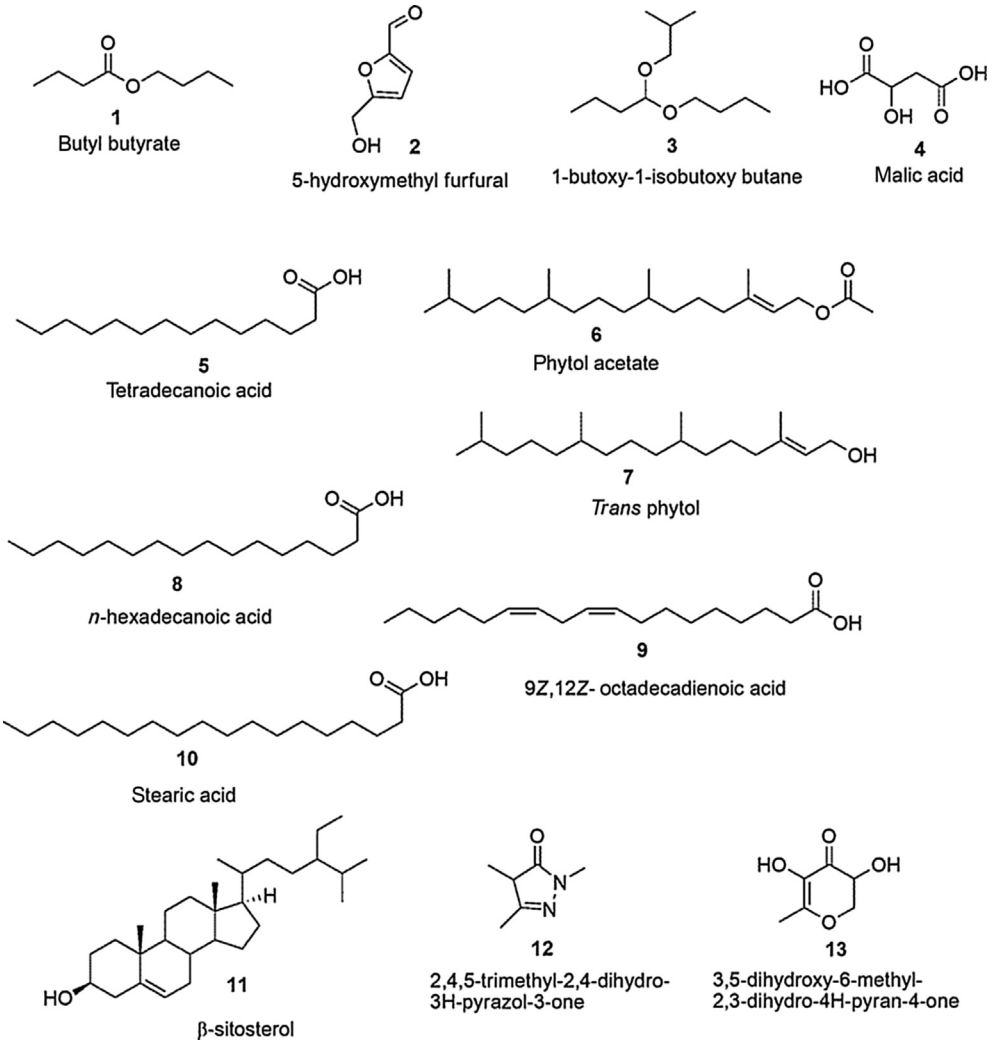

Figure 5 – Chemical structures of compounds identified in the ethanolic extract of *Ficus carica* L. fruit by GC–MS. GC–MS = gas chromatography–mass spectroscopy.

(Figure 1, Table 2). Similar antioxidant activities were also reported for the methanol extracts and ethyl acetate fraction of *Ficus microrpa* leaf, bark, and fruit in a previous study [32]. Generally, oxidative stress reveals an imbalance between systemic manifestations of reactive oxygen species and the inability of physiological system to detoxify the reactive intermediates and free radicals [33]. Free radicals damage all components of cells including DNA, proteins, and lipids and the destruction of cells in the normal redox state can cause toxic effects through the production of peroxides [34]. There has been a growing interest in the use of potential antioxidants for medicinal purposes, particularly for the oxidative stress related metabolic disorders [35]. The higher antioxidant activity of the different bioactive compounds in *F. carica* fruit ethanolic extract also proved it to be a potential source of free radical scavenging antioxidants. The 2,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, 5-hydroxymethyl furfural, and malic acid are identified compounds in *F. carica* and have been reported as potent antioxidants in some very recent and previous studies [36,37]. Therefore, the potent antioxidant activity of the fruit ethanolic extract of *F. carica* might be partly contributed to its antidiabetic and antiobesogenic activities which have been confirmed by the inhibition of some relevant enzymes.

Significant inhibition of carbohydrate digesting enzymes such as α-glucosidase and α-amylase, suggest that phytochemicals present in the fruit of *F. carica* could be lead compounds for the treatment of T2D. Two enzymes, α-glucosidase and α-amylase, are involved in the hydrolysis of polysaccharides and disaccharides to simple sugars [35]. The inhibition of these enzymes may delay the process of carbohydrate digestion and the release of glucose molecules into the blood [35]. In a recent study, Gou et al [38] reported the strong and dose-dependent noncompetitive inhibitory effect of malic acid on α-glucosidase enzyme activity *in vitro* which has been further confirmed by computation dynamics study. In our study, malic acid was identified as one of the compounds in *F. carica* fruit extract and this compound might work as a potential noncompetitive inhibitor of α-glucosidase enzyme (Figure 3). Additionally, chemical compounds such as *n*-hexadecanoic acid, trans phytol, and 5-hydroxymethylfurfural found in *F. carica* fruit extract have

Table 3 – Phytochemical compounds identified in the ethanolic extract of *Ficus carica* fruit by GC–MS.

No.	Compounds	Retention time (min)	Mass (amu)	Peak area (%)
1	Butyl butyrate	5.25	144 [M^+]	0.24
2	5-Hydroxymethyl furfural	8.41 and 8.68	126 [M^+]	1.94
3	1-Butoxy-1-isobutoxy butane	8.62	159 [$M^+ - C_3H_7$]	1.94
4	Malic acid	9.10	116 [$M^+ - H_2O$]	0.44
5	Tetradecanoic acid	14.94	228 [M^+]	0.29
6	Phytol acetate	15.74	278 [$M^+ - acyl - H_2O$]	85.86
7	*trans* Phytol	16.19	278 [$M^+ - H_2O$]	0.67
8	*n*-Hexadecanoic acid	17.03	256 [M^+]	1.83
9	9Z12Z-Octadecadienoic acid	18.65	280 [M^+]	0.48
10	Stearic acid	18.92	284 [M^+]	0.39
11	Sitosterol	29.46	414 [M^+]	1.06
12	245-Trimethyl-24-dihydro-3*H*-pyrazol-3-one	6.50	126 [M^+]	0.98
13	35-Dihydroxy-6-methyl-23-dihydro-4*H*-pyran-4-one	7.43	144 [M^+]	0.60

been shown to have antidiabetic and antiobesity activity in some previous studies [39–41]. Therefore, *F. carica* fruit extract might show its antidiabetic activity not only by reducing oxidative stress but also by inhibiting α-glucosidase enzyme activity mediated by some of its bioactive compounds.

Pancreatic lipase is the most active enzyme in the digestion of dietary fats and enables absorption into the intestinal lumen [42]. Lipase inhibition is one of the key regulatory targets in obesity. Inhibition of pancreatic lipase by the ethanolic extract of the fruit of *F. carica* L. could be a promising lead for the treatment of obesity when some compounds found in our extract might be responsible for this effect. In a previous study, malic acid containing extracts of *Phellinus linteus* showed strong lipase inhibitory activity *in vitro* [43]. Chai et al [44] reported the potential benefits of sitosterol against hormone sensitive lipase, adipogenesis, and lipolysis mechanism in rat adipocytes. The fruit extract of edible Tunisian *F. carica* reported the *in vivo* antihyperlipidemic activity [24], which is in line with the results of our current study, particularly in terms of antilipase activity of *F. carica* fruit ethanolic extract.

5. Conclusion

The data of this study suggest that the fruit ethanolic extract of *F. carica* L. could be a potential alternative for the treatment of oxidative stress, T2D, and obesity, However, further studies on humans and experimental animals are needed to confirm these effects.

Conflicts of interest

The authors have no conflict of interest to declare within this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2017.03.001.

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