Comparative DNA profiling, phytochemical investigation, and biological evaluation of two *Ficus* species growing in Egypt

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ABSTRACT

Aim and Background: A comparison between two *Ficus species*, cultivated in Egypt, was carried out in this study. Their DNA analysis revealed that they are not closely related. **Materials and Methods:** The pharmacopoeial constants of the leaves showed higher total ash and acid insoluble ash in *F. lyrata* than in *F. platypoda*. The other parameters were close in both species. Preliminary phytochemical screening revealed the presence of carbohydrate and/or glycosides, tannins, flavonoids, sterols, and triterpenes in their leaves and was detected in traces in their stems. **Results:** Saponification of n-hexane extract of the leaves yielded 46% and 74.8% for the unsaponifiable matters and 20% and 15% for the fatty acids for *F. platypoda* and *F. lyrata*, respectively. n-Docosane (21.69%) and n-heptacosane (33.77%) were the major hydrocarbons in F. platypoda and *F. lyrata*, respectively. b-Sitosterol was the main sterol, palmitic (22.07%) and carboceric (35.72%) acids were the major identified saturated fatty acids in both species, while linoleic acid was the main unsaturated fatty acid (18.66% and 16.7%) in both species, respectively. The acute toxicity study revealed that the two species were safe up to 2 g/kg. The antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and pyrogallol as the standard was more significant for *F. platypoda* (232.6 μg/ml) than for *F. lyrata*, (790.9 μg/ml). The oral antihyperglycemic activity in diabetic rats using alloxan revealed that the 80% ethanolic extract of the leaves of *F. platypoda* was more active than that of the leaves of *F. lyrata* in decreasing the blood glucose level at 200 mg/kg/day (107.9 ± 5.817, 127.2 ± 4.359) and 400 mg/kg/day (64.11 ± 4.358, 127.7 ± 6.889), respectively, when compared with the diabetic control gliclazide (172.3 ± 2.089). **Conclusion:** The results of this study provide evidence that the two *Ficus species* have antioxidant and antihyperglycemic activity, in the order *F. platypoda* and then *F. lyrata*

Key words: Acute toxicity, antihyperglycemic, antioxidant, DNA, *Ficus platypoda*, *Ficus lyrata*

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INTRODUCTION

Family Moraceae is one of the biggest families that contain around 2000 species of woody plants, trees, erect shrubs, and climbers related to genus *Ficus*. The latter is known as fig trees or figs. They are grown for their fragrance and ornament. They are native throughout the tropics with a few species extending into the semi-warm temperate zone.[1,2] *Ficus* species have been long used worldwide as astringent, carminative, vermicide, hypotensive, anthelmintic, and antidysentry agents.[3] In Egypt, several *Ficus* species are used in folk medicine as antidiabetic, hypotensive, and anticough agents, as well as in certain skin diseases treatment.[3] It was reported that some *Ficus* species can be used as a remedy for visceral obstructive disorder, leprosy, and respiratory disorders.[3] *Ficus platypoda* (Miq) A.Cunn, known as desert fig or rock fig, is endemic to central, northern Australia, and Indonesia.[4] On the other hand, *Ficus lyrata* Warb, known as *Ficus pandurata* Sander or Chinese fig, with fiddle-shaped

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or banjo-shaped leaves, is indigenous to tropical, central, and west Africa. It is used as a shade tree and is suitable for indoor growing.[2]

Few reports showed the chemical constituents and biological activities of *F. lyrata*.[5,6] However, nothing could be traced concerning *F. platypoda*. This encouraged the authors to run a comparative study of the chemical composition and biological activities of both species cultivated in Egypt.

MATERIALS AND METHODS

Plant material

Samples of *F. platypoda* (Miq) A. Cunn. and *F. lyrata* Warb. used in this study were collected in march 2009 from Giza Zoo, Cairo, Egypt. The plants were kindly identified by Dr. Mohamed Gibali, Senior Botanist. Voucher specimens of both species were deposited in Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University under the registration numbers 2009BUPD18 and 2009BUPD19 respectively.

Leaves and stems of both species were air-dried, powdered, and stored for chemical and biological studies. For DNA profiling, fresh leaves were freeze-dried and ground under liquid nitrogen to fine powder.

Preparation of the extracts

The air-dried powdered leaves of both species (500 g each) were exhaustively extracted with 80% ethanol and the solvent was evaporated under reduced pressure. The residues obtained were kept for biological study.

For the investigation of lipoidal matter content, the powdered leaves of both species (25 g each) were extracted via maceration in *n*-hexane (3 × 100 ml each) until exhaustion. The residues left after evaporation of the solvent were kept for analysis of the unsaponifiable (USM) and saponifiable matters.

DNA profiling

DNA profiling refers to the use of a technique based on polymerase chain reaction (PCR, a system for the amplification of DNA) to reveal the specific DNA profile for both species.

Eleven primers, purchased from Operon Technologies Inc. (Almeda, California, USA), were used for random amplified polymorphic DNA (RAPD) with the following sequences: OPB-03 5'-CATCCCCCTG-3', OPG-02 5'-GGCACTGAGG-3', OPA-09 5'-GGGTAACGCC-3', OPC-01 5'-TTCGAGCCAG-3', OPC-19 5'-GTTGCCAGCC-3', OPM-05 5'-GGGAACGTGT-3', OPB-17 5'-AGGGAACGAG-3', OPA-06 5'-GGTCCCTGAC-3', OPE-05 5'-TCAGGGAGGT-3', OPC-12 5'-TGTCATCCCC-3', OPM-01 5'-GTTGGTGGCT-3'.

DNA extraction and quantification

DNA was extracted using the cetyl trimethylammonium bromide (CTAB) method of Doyle and Doyle.[7] Sample of frozen leaves (50 mg each) were powdered in liquid nitrogen, separately extracted with 0.8 ml CTAB, precipitated with isopropanol, washed in 70 % ethanol, and dissolved in deionized water.

Amplification of random amplified polymorphic DNA-PCR markers

Eleven oligonucleotide primers of arbitrary sequences were used in this study. The PCRs were carried out with 100 ng of genomic DNA template following a thermal cyclic program using Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems). Amplified products were analyzed by electrophoresis in 2 % agarose gels [A Gibco BRL Life Technologies (Paisely, UK) agarose gel] and finally stained with ethidium bromide. A molecular size marker was used as a standard marker.

Analysis of RAPD data

RAPD bands were treated as presence or absence, without considering their percentage. For estimating genetic distance among the tested samples, each DNA band was treated as a unit character. The genetic similarity coefficient (GS) between two genotypes was estimated according to the equation of Jaccard.[8] GS = 2Nab/(Na+Nb), where Nab is the number of scored fragments between plants a and b; Na is the number of scored fragment.

Phytochemical characterization

Determination of pharmacopoeial constants of the leaves of both species

Certain pharmacopoeial constants of the dried powdered leaves of both species were determined according to the Egyptian Pharmacopoeia, 2005.[9] These include total ash, acid insoluble and water soluble ashes as well as crude fiber, and moisture contents.

Phytochemical screening

Phytochemical screening for the major chemical constituents was conducted using standard qualitative methods.[10-12] The leaves and stems of both species under investigation were screened for the presence of crystalline sublimate, steam volatile substances, carbohydrates and/or glycosides, tannins, flavonoids, saponins, sterols and/or triterpenes, alkaloids, coumarins, anthraquinones, and cardiac glycosides.

Study of the lipoidal content

Preparation of USM and saponifiable matter

The *n*-hexane extract of the leaves of both species (0.5 g, each) was separately saponified[13] by reflux overnight with 5 ml of 10% alcoholic KOH at room temperature. The USM, in each case, was extracted with ether and saved for gas chromatography (GC) analysis. The remaining aqueous solutions were then acidified with dilute HCl (10%) and extracted with ether till exhaustion and saved for analysis of the fatty acids (FA).[13]

Preparation of the fatty acid methyl esters (FAMEs)

Each of the fatty acid mixtures was separately dissolved in 1 ml methanol, and 0.5 ml of sulphuric acid was added. The mixture, in each case, was treated according to Finar (1973),[14] and the obtained FAME was subjected to GC analysis.

Gas liquid chromatography (GLC) analysis of the USM and FAME

USM analysis was carried out on Hewlett-Packard HP-6890 N network GC system equipped with a FID detector. Analysis was performed on an HP-5 column using N_2 as the carrier gas, injection temperature 250°C, detector temperature 300°C. Aliquots, 2 μL each, of 2% chloroformic solutions of the analyzed USM and reference samples were co-chromotographed. Identification of the components was based on comparison of the retention times of their peaks with those of the available authentic samples. The relative amount of each component was calculated via peak area measurement by means of computing integrator.

FAMEs were analyzed on a pye Unicam 304 series GC equipped with a FID detector. Analysis was performed on 10 % PEGA (on chromosorb W-AW, 100-120 mesh) column using N_2 as the carrier gas, injection temperature 250°C, detector temperature 280°C. Aliquots, 2 μL each, of 2 % chloroformic solutions of the analyzed FAME and reference FAMEs were co-chromatographed. Identification of the FA was carried out by comparing the retention times of their methyl esters with those of the available reference FA_s similarly analyzed.

Biological study

Experimental animals

Mature albino mice (weight 20–25 g) of both sexes were used for acute toxicity study, and adult male albino Sprague–Dawely rats (weight 130–160 g, age 10–12 weeks) were used for the other investigated biological activities. These animals were obtained from the animal house colony of the National Research Center (NRC), Egypt. Animal experiments were performed in accordance with national guidelines for the use and care of laboratory animals. The animals were housed in colony cages under standard light, temperature, and room humidity conditions for at least 2 weeks before the experimental sessions. All animal procedures were performed after approval from the Ethics Committee of NCR, Egypt and in accordance with the recommendations of the proper care and use of laboratory animals.

Kits, drugs, and devices

Kits, purchased from Biodiagnostic Company (Egypt) were used for determination of glucose, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), and hemoglobin (Hb). Alloxan monohydrate was obtained from Sigma Chemical Company (USA) and gliclazide standard was obtained from Servier Egypt Industries and Double beam spectrophotometer (UV-150-02) Shimadzu, Japan, for the biological assays.

Acute toxicity study in mice

Acute oral toxicity study of 80 % ethanol extracts of the leaves of *F. platypoda* and *F. lyrata* were determined in mice according to Lorke (1983).[15] Animals were observed for 24 h for any sign of toxicity or death. Two weeks later, blood samples from the retro-orbital plexus of all mice were obtained, for estimation of blood Hb, red blood cell counts (RBCs), and total leukocytes count (TLC).

Antihyperglycemic activity

The rats were rendered diabetic after a single intraperitoneal injection of alloxan monohydrate in a dose of 150 mg/kg body weight and then anesthetized by ether, and blood samples were collected from the retro-orbital venous plexus for glucose level determination. The rats were divided into seven groups of 6 rats each and were treated as follows: Group I: negative control; Group II: diabetic non-treated rats as positive control; Group III and IV: diabetic rats, orally treated with 80 % ethanolic extracts of *F. platypoda* at two doses (200 and 400 mg/kg body weight), respectively; Group V and VI: diabetic rats, orally treated with 80 % ethanol extracts of *F. lyrata* at two doses (200 and 400 mg/kg body weight), respectively. All previous doses represent 1/10 and 1/5 of the maximum soluble concentration. Group VII: diabetic rats were treated with a single oral dose (20 mg/kg body weight) of a standard antidiabetic drug (gliclazide). Drugs were administered for 28 days, blood samples were then collected for measurement of biochemical parameters.

Biochemical analysis

Determination of serum glucose level was carried out according to the method described by Joy and Kuttan.[16] The plasma total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol were quantified using enzymatic kits.[17]

Antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed according to the method of Amic *et al.*,[18] and Phang *et al.*,[19] using 80 % ethanol extracts of *F. platypoda* and *F. lyrata* leaves at different concentrations (200 and 400 mg/kg body weight). The absorbance of the reaction mixtures was measured at 520 nm. Methanol was used as a blank, methanolic solution of pyrogallol as a standard, and the EC_{50} values were calculated.

Statistical analysis

Analysis of the data was performed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Statistical analysis was performed using SPSS (Version 14). A level of P values <0.05 was considered to be significant.

RESULTS AND DISCUSSION

The DNA profiling is generally independent of environment and is consistent throughout different parts and developmental stages of growth. Similarity of DNA fingerprints depends on the genetic closeness of the tested samples.[20] The extracted DNA of each of the two *Ficus* species was amplified using 11 decamer primers to detect their genetic variability. Each of the 11 primers had successfully directed the amplification of a genome-specific fingerprint of DNA fragments and consequently serves to evaluate interspecific diversity between both species. Such primers could be used to discriminate between the two *Ficus* species depending on their low values of similarity coefficients and high level of polymorphism.

Table 1: Total number of RAPD-PCR fragments, distribution of monomorphic and polymorphic bands, percentage of polymorphic fragments, and similarity coefficient generated by 11 decamer arbitrary primers in *Ficus platypoda* (Miq) A.Cunn and *Ficus lyrata* Warb

Primer codes	RAPD fragments		Monomorphic fragments	Polymorphic fragments	% of polymorphic fragments	Similarity coefficient
	L	P				
OPB-03	11	11	8	6	27.27	72.72
OPB-17	16	13	8	13	44.82	55.17
OPE-05	8	10	5	8	44.44	55.55
OPG-02	10	13	7	9	39.13	60.86
OPA-06	12	8	6	8	40	60
OPA-09	12	12	6	12	50	50
OPC-01	11	15	9	8	30.76	69.23
OPC-12	14	13	8	11	40.74	59.25
OPC-19	13	11	7	10	41.66	58.33
OPM-01	11	12	8	7	30.43	69.56
OPM-05	16	12	7	14	50	50
Total	134	130	79	106	39.93	60.06

P: *F. platypoda*, L: *F. lyrata*, RAPD: Random Amplified Polymorphic DNA

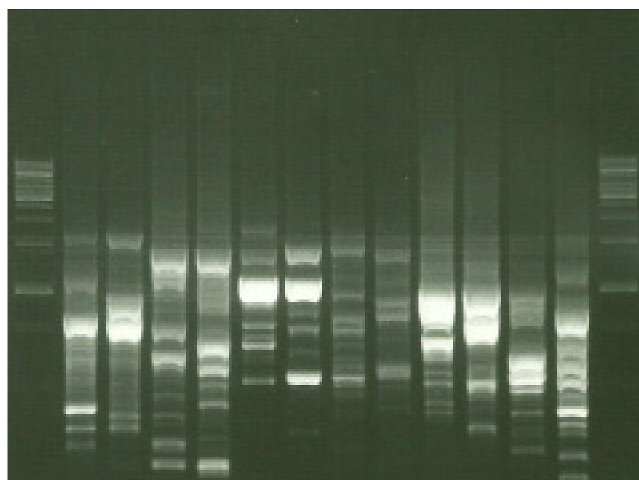

Figure 1a: RAPD-PCR products for *Ficus platypoda* (Miq) A. Cunn and *Ficus lyrata* Warb using six decamer primers (OPB-03, OPB-17, OPB-05, OPG-02, OPA-06, OPA-09)

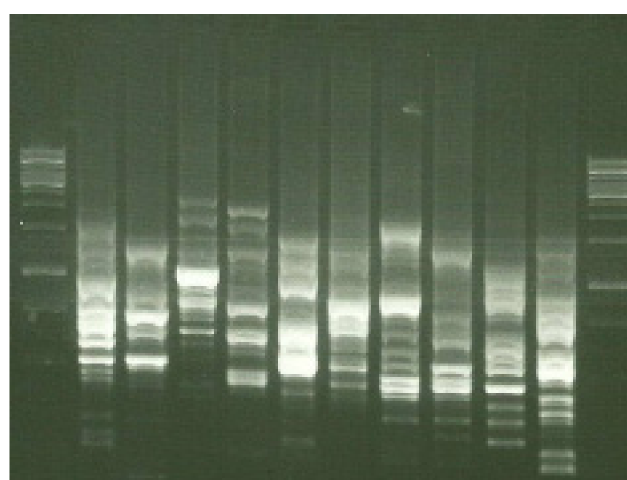

Figure 1b: RAPD-PCR products for *Ficus platypoda* (Miq) A. Cunn and *Ficus lyrata* Warb using five decamer primers (OPC-01, OPC-12, OPC-19, OPM-01, OPM-05)

The obtained RAPD-PCR products of the two *Ficus* species are shown in Table 1 and are represented in Figures 1a and b. The 11 primers of arbitrary sequences generated 130 fragments in *F. platypoda* and 134 fragments in *F. lyrata*.

The 11 primers produced multiple band profiles with a number of amplified DNA fragments of 16 when OPM-05 and OPB-17 were used in *F. lyrata*. On the other hand, the least number of fragments was 8, being produced by OPE-05 in *F. lyrata* and OPA-06 in *F. platypoda*.

The total number of fragments was 264 bands, 106 were polymorphic, representing a level of polymorphism of 39.93%. The highest degree of similarity (72.72%) was recorded using primer OPB-03, and the lowest degree of similarity (50%) was recorded using primers OPA-09 and OPM-05 as shown in Table 1. The low percentage of similarity coefficients indicates that the two species are not closely related and this difference in genetic profile may influence the chemical composition that affect their therapeutic activity.

From the previous findings, it was obvious that the most relevant fragment resulting from successful combination of template and primer was that produced by OPA-09, OPM-05 RAPD primers.

Table 2: Pharmacopoeial constants of the leaves of *Ficus platypoda* (Miq) A. Cunn and *Ficus lyrata* Warb

Species	*Ficus platypoda* %	*Ficus lyrata*
Total ash	13.10	20.10%
Acid insoluble ash	8.10	13.90%
Water soluble ash	2.10	1.60%
Crude fibre	19.35	20.00%
Moisture	6.00	5.50%

The percentage of the total ash and acid insoluble ash were found to be higher (20.1 % and 13.1 %) in the leaves of *F. lyrata* than in those of *F. platypoda* (13.9 % and 8.1 %, respectively), while the percentage of water-soluble ash, crude fiber, and moisture content were close in both species [Table 2]. The variation in total and acid insoluble ashes could be used as a valuable criterion for discrimination between the two species. The high total ash values obtained for both species, especially, *F. lyrata* indicate the presence of carbonates, phosphates, silicates, and silica,[9] which is derived from the plant tissue itself (physiological ash), and the residue of the adhering material to the plant, e.g., sand and soil (non-physiological ash)

Preliminary phytochemical investigation revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, sterols, and triterpenes in the leaves of both species and in trace amount in both stem samples. Coumarins were found in traces in the leaves of both species. Crystalline sublimate, steam volatile substances, saponins, alkaloids and/or nitrogeneous bases, anthraquinones, and cardiac glycosides were absent from both species [Table 3]. These findings showed the great similarities in total chemical contents of both species, but still may have differences in the individual bioactive contents that may be responsible for the biological activities as will be shown later.

Saponification of *n*-hexane extracts of the leaves of both species yielded 46 % and 74.8 % for USM and 20 % and 15 % for FA of *F. Platypoda* and *F. lyrata,* respectively. The percentage of total identified hydrocarbons, sterols and triterpenes were higher in *F. lyrata* (55.73 and 26.37%, respectively) than in *F. platypoda* (53.74 and 15.57%, respectively). *n*-Docosane was the main hydrocarbon

Table 3: Preliminary phytochemical screening of the leaves and stems of *Ficus platypoda* (Miq) A. Cunn and *Ficus lyrata* Warb

Chemical test		*Ficus platypoda*		*Ficus lyrata*	
		Leaves	Stem	Leaves	Stem
Crystalline sublimate;		-	-	-	-
Steam volatile substances		-	-	-	-
Carbohydrates and/or glycosides		+	+	+	+
Tannins	• Condensed	+	±	+	±
	• Hydrolysable	+	±	+	±
Flavonoids	• Free	+	±	+	±
	• Combined	+	±	+	±
Saponins		-	-	-	-
Sterols and /or triterpenes		+	±	+	±
Alkaloids		-	-	-	-
Coumarins		±	-	±	-
Anthraquinones	• Free	-	-	-	-
	• Combined	-	-	-	-
Cardiac glycosides		-	-	-	-

(+): present, (-): absent, (±): traces

(21.69%) in *F. platypoda,* while *n*-heptacosane was the major one (33.77%) in *F. Lyrata* [Table 4]. The percentage of the total identified, saturated, and unsaturated FA were higher in *F. lyrata* (97.46, 63.35, and 34.35%) than in *F. platypoda* (84.05, 51.5, and 32.55%). Palmitic was the major saturated FA in *F. platypoda* (22.07%), and carboceric acid was the major saturated FA in *F. lyrata* (35.72%), while linoleic acid was the predominant usaturated FA in *F. platypoda* (18.6%) and *F. Lyrata* (16.70%) [Table 5]. Linolenic acid (6.74%) was detected only in *F. platypoda*. These polyunsaturated FA (PUFAs) cannot be made in the body and must be provided by diet and are known as essential FA. Within the body, they can be converted to other PUFAs such as arachidonic acid, eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA), which are important for maintaining the membranes of all cells; production of prostaglandins that regulate many body processes include inflammation and blood clotting. On the other hand, they play very important role to enable the fat-soluble vitamins A, D, E, and K to be absorbed from food and in regulating body cholesterol metabolism.[21]

The results of acute toxicity studies revealed that 80% ethanol extracts of the leaves of both species using the tested dose, 2 g/kg, showed no significant difference from the normal control in Hb, RBCs, and TLC [Table 6]. None of the extracts produced any apparent behavioral changes,

Table 4: Components identified by GLC analysis of the unsaponifiable matters (USM) of the leaves of *Ficus platypoda* (Miq) A. Cunn and *Ficus lyrata* Warb leaves

Peak no	Identified components	RR_t^*	Area %	
			F. platypoda	*F. lyrata*
1	*n*-Octadecane	0.65	0.35	0.94
2	*n*-Eicosane	0.7	2.03	4.01
3	*n*-Heneicosane	0.76	0.92	-
4	*n*-Docosane	0.78	21.69*	-
5	*n*-Tricosane	0.82	1.83	-
6	*n*-Tetracosane	0.84	6.17	4.54
7	α-Amyrin	0.89	3.01	-
8	*n*-Pentacosane	0.9	-	2.35
9	*n*-Hexacosane	0.92	7.10	8.77
10	*n*-Heptacosane	1.00	11.13	33.77*
11	*n*-Octacosane	1.04	1.30	1.35
12	*n*-Nonacosane	1.07	1.22	-
13	Bicadinane	1.12	1.30	17.16
14	Campasterol	1.25	0.238	2.03
15	Stigmasterol	1.3	5.06	2.09
16	*β*-Sitosterol	1.37	5.96*	5.09
Total identified components			70.61%	82.10%
Identified hydrocarbons			53.74%	55.73%
Identified sterols and triterpenes			16.9%	26.37%

RR_t^* = Retention time relative to *n*-heptacosane (R_t = 24.8 min), *: Major component

Table 5: Components identified by GLC analysis of fatty acid methyl esters (FAME) of the leaves of *Ficus platypoda* (Miq) A. Cunn and *Ficus lyrata* Warb

Peak no	Identified Components	RR_t^*	Area %	
			F. platypoda	*F. lyrata*
1	Myristic acid (C_{14})	0.61	2.08	-
2	Palmitic acid (C_{16})	0.78	22.07*	4.39
3	Palmetoleic acid ($C_{16:1}$)	0.84	0.92	-
4	Stearic acid (C_{18})	0.93	5.45	-
5	Oleic acid ($C_{18:1}$)	0.95	5.49	2.41
6	Linoleic acid ($C_{18:2}$)	1	18.66*	16.7*
7	Linolenic acid ($C_{18:3}$)	1.06	6.74	-
8	Behenic acid (C_{22})	1.22	2.57	20.91
9	Erucic acid ($C_{22:1}$)	1.25	0.53	3.45
10	Lignoceric acid (C_{24})	1.35	10.82	2.33
11	Hervonic acid ($C_{24:1}$)	1.36	0.21	11.55
12	Carboceric acid (C_{27})	1.62	8.51	35.72*
Total identified components			84.05%	97.46%
Total saturated fatty acid			51.50%	63.35%
Total unsaturated fatty acid			32.55%	34.11%

RR_t^* = Retention time relative to linoleic acid (R_t = 20.75 min),*: Major component

Table 6: Effect of 80% ethanol extracts of *Ficus lyrata* Warb and *Ficus platypoda* (Miq) A. Cunn. Leaves on hemoglobin (Hb), red blood cell count (RBCs) and total leukocytic count (TLC) in mice

Groups	Dose (g/kg) p.o.	Hemoglobin Conc. (g/dl)	Red Blood Cells (10^6/mm^3)	Total Leukocytic Count (10^3/Mm^3)
Normal control	Saline	11.18 ± 0.13	3.786 ± 0.032	5.140 ± 0.28
Ficus platypoda	2	10.16 ± 0.25	3.400 ± 0.060	4.900 ± 0.14
Ficus lyrata	2	10.90 ± 0.23	3.650 ± 0.073	4.860 ± 0.16

Values represent the mean ± S.E. of five mice for each group. No significant difference from the control (one way ANOVA, $P < 0.05$)

Table 7a: Effect of 80% ethanol extracts of *Ficus platypoda* (Miq) A. Cunn. and *Ficus lyrata* Warb leaves on serum cholesterol and triglycerides levels in rats

Groups	Dose/ p.o	Cholesterol (mg/dl)			Triglycerides (mg/dl)		
		Basal	After 72 h	After 1 month	Basal	After 72 h	After 1 month
Normal control	Saline	169.7 ± 6.445	166.4 ± 5.198	156.3 ± 4.180	94.41 ± 4.369	98.31 ± 3.867	102.3 ± 4.943
Diabetic control	Saline	169.4 ± 2.244	186.2 ± 7.583	170.5 ± 5.117	98.41 ± 4.330	171.5 ± 6.056*	109.3 ± 3.047
F. platypoda	200 mg/kg/day	180.3 ± 3.967	177.7 ± 2.229	169.7 ± 4.147	105.3 ± 2.467	170.6 ± 9.737*	109.4 ± 4.796
	400 mg/kg/day	174.4 ± 3.918	180.4 ± 3.412	162.8 ± 5.131	94.23 ± 5.492	169.2 ± 7.510*	113.4 ± 6.697
F. lyrata	200 mg/kg/day	185.3 ± 2.798	185.6 ± 2.358	175.7 ± 5.589	95.56 ± 1.483	175.3 ± 8.547*	89.61 ± 3.470@
	400 mg/kg/day	182.3 ± 3.516	183.2 ± 3.386	163.0 ± 7.922	94.01 ± 3.330	169.5 ± 3.010*	81.80 ± 1.686*@
Gliclazide	20 mg/kg/day	180.6 ± 3.792	186.6 ± 5.718	159.1 ± 6.610	95.27 ± 4.627	175.6 ± 4.369*	81.93 ± 3.724*@

Values represent the mean ± S.E. of seven rats for each group, *$P < 0.05$: Statistically significant from normal control, @$P < 0.05$: Statistically significant from diabetic control, p.o: orally

Table 7b: Effect of 80% ethanol extracts of *Ficus platypoda* (Miq) A. Cunn. and *Ficus lyrata* Warb leaves on serum HDL and LDL levels in rats

Groups	Dose/Route of administration	HDL (mg/dl)			LDL (mg/dl)		
		Basal	After 72 h	After 1 month	Basal	After 72 h	After 1 month
Normal control	Saline	65.06 ± 2.549	61.84 ± 0.9489	53.09 ± 0.9760	85.30 ± 6.067	85.41 ± 6.484	82.79 ± 3.810
Diabetic control	Saline	63.54 ± 3.515	44.91 ± 0.8626*	41.81 ± 1.786*	86.17 ± 3.513	107.0 ± 7.552	106.8 ± 5.477
F. platypoda	200 mg/kg/day	70.34 ± 3.176	45.93 ± 1.570*	71.06 ± 1.874*@†	88.91 ± 2.725	103.8 ± 7.281	76.76 ± 5.518*
	400 mg/kg/day	66.66 ± 3.446	46.46 ± 2.223*	66.56 ± 1.032*@†	88.86 ± 5.570	111.0 ± 7.153	73.51 ± 4.802*
F. lyrata	200 mg/kg/day	74.94 ± 1.581	49.41 ± 1.998*	68.54 ± 2.370*@†	88.63 ± 2.508	104.5 ± 2.932	89.21 ± 4.434
	400 mg/kg/day	71.19 ± 2.510	50.24 ± 1.244*	66.11 ± 1.080*@†	86.21 ± 4.436	103.1 ± 4.371	80.51 ± 8.235*
Gliclazide	20 mg/kg/day	71.26 ± 1.979	45.29 ± 3.331*	51.66 ± 6.716	89.67 ± 3.607	106.2 ± 5.375	91.10 ± 5.439

Values represent the mean ± S.E. of seven rats for each group, *$P < 0.05$: Statistically significant from normal control, @$P < 0.05$: Statistically significant from diabetic control, †$P < 0.05$: Statistically significant from gliclazide, p.o: per oral

Table 8a: Free radical scavenging activity of 80% ethanol extract of leaves of *Ficus platypoda* (Miq) A. Cunn. and *Ficus lyrata* Warb at different concentrations

Conc. (µg/ml)	Radical scavenging activity (%) of *Ficus platypoda*	Radical scavenging activity (%) of *Ficus lyrata*
10000	7.79	49.22
2500	70.72	84.42
625	84.42	40.50
156.25	36.14	11.53
39.06	17.76	-19.63
9.77	12.15	-1.87
2.44	2.80	-4.67
0.61	1.25	-2.18

Table 8b: Free radical scavenging activity of Pyrogallol as a positive control at different concentrations

Conc. (µg/ml)	Radical scavenging activity (%) of Pyrogallol
1000	89.54
200	88.19
40	61.58
8	2.69
1.6	−10.01
0.32	−3.29

and there was no mortality as observed up to 24 h in all tested doses. Based on these results, 1/10 and 1/5 of the maximum soluble concentrations (200 and 400 mg/kg body weight) were selected for pharmacological studies of

Table 9: Effect of 80% ethanol extracts of *Ficus platypoda* (Miq) A. Cunn. and *Ficus lyrata* Warb leaves on blood glucose level in rats

Groups	Dose / p.o	Glucose (mg/dl)		
		Basal	After 72 h	After 1 month
Normal control	Saline	93.09 ± 4.626	109.0 ± 3.710	112.5 ± 3.620@
Diabetic control	Saline	98.70 ± 5.636	246.4 ± 10.56*	172.3 ± 2.089*†
F. platypoda	200 mg/kg/day	99.54 ± 6.416	256.1 ± 10.12*	107.9 ± 5.817@
	400 mg/kg/day	99.53 ± 4.979	261.8 ± 15.63*	64.11 ± 4.358*@†
F. lyrata	200 mg/kg/day	103.8 ± 8.262	252.1 ± 14.35*	127.2 ± 4.359@
	400 mg/kg/day	104.8 ± 9.699	242.3 ± 10.13*	127.7 ± 6.889@
Gliclazide	20 mg/kg/day	101.8 ± 4.233	237.8 ± 8.486*	110.8 ± 7.240@

Values represent the mean ± S.E. of seven rats for each group, *$P < 0.05$: Statistically significant from normal control, @$P < 0.05$: Statistically significant from diabetic control, †$P < 0.05$: Statistically significant from gliclazide, p.o: orally

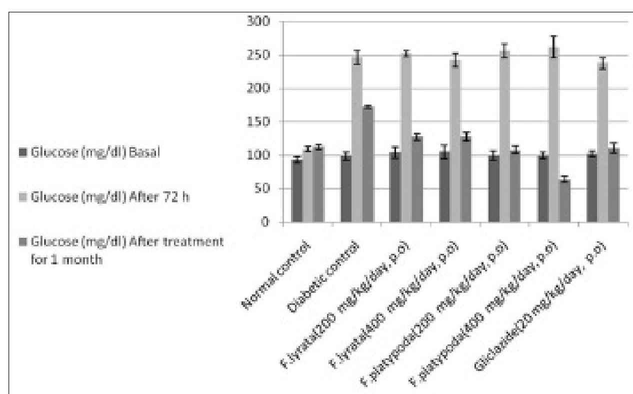

Figure 2: Effect of 80% ethanol extracts of *Ficus lyrata* Warb and *Ficus platypoda* (Miq) A.Cunn. leaves on blood glucose level in rats

both samples. Both extracts showed a significant increase in HDL and a significant decrease in LDL and no significant effect on cholesterol and TG_S levels [Tables 7a and 7b]. These results could be related to the presence of high content of PUFAs in the two species.

The ethanol extract of *F. platypoda* at different concentrations (0.61, 2.44, 9.77, 39.06, 156.25, 625, 2500, and 10000 μg/mL) showed marked antioxidant activity. The scavenging activities were 1.25, 2.8, 12.15, 17.76, 36.14, 84.42, 70.72, and 7.79%, respectively, and the EC_{50} was calculated to be 232.6 μg/ml. On the other hand, the ethanol extract of *F. lyrata* showed marked antioxidant activity at higher concentrations (156.25, 625, 2500, and 10000 μg/mL). The scavenging activities were 11.53, 40.50, 84.42, and 49.22%, respectively, and the EC_{50} was calculated to be 790.9 μg/ml as shown in Table 8a and 8b. Results showed that the ethanol extract of leaves of both species have antioxidant activities, especially *F. platypoda*, compared to pyrogallol as a positive control. These results agree with those previously reported for *F. lyrata*.[6] The antioxidant activity of the investigated species may be attributed to their flavonoid content that play a vital role in preventing health disorders related to oxidative stress, including vascular diseases, neurgenerative diseases, and cancer.[22]

Results of antihyperglycemic activity showed that 80% ethanol extract of the leaves of *F. platypoda* produced a significant reduction in blood glucose level (107.9 ± 5.817, 64.11 ± 4.358) at the tested doses 200, 400 mg/kg/day, respectively, in comparison with *F. lyrata* (127.2 ± 4.359, 127.7 ± 6.889) as well as the standard hypoglycemic drug gliclazide (110.8 ± 7.240) after 1 month of treatment [Table 9, Figure 2].

CONCLUSION

Considering that the two species are not closely related, their chemical composition and thus their therapeutic activity may not be the same. The results of this study provide evidence that the two *Ficus* species have antioxidant and antihyperglycemic activity, in the order *F. platypoda* and then *F. lyrata*. Therefore, these species may have great relevance in the prevention and therapies of diseases in which oxidants are implicated after further *in vitro* and *in vivo* studies for understanding their mechanism of action as antioxidant. Also, these plants can be good candidates for further phytochemical and chromatographic studies for isolation and identification of the compounds responsible for these antioxidant and antihyperglycemic activities.

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