Chemotaxonomic Diversity of Three Ficus Species: Their Discrimination Using Chemometric Analysis and Their Role in Combating Oxidative Stress

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Submitted: 25-12-2016 Revised: 01-02-2017 Published: 11-10-2017

ABSTRACT

Background: Genus Ficus (Moraceae) constitutes more than 850 species and about 2000 varieties and it acts as a golden mine that could afford effective and safe remedies combating many health disorders. Objectives: Discrimination of Ficus cordata, Ficus ingens, and Ficus palmata using chemometric analysis and assessment of their role in combating oxidative stress. Materials and Methods: Phytochemical profiling of the methanol extracts of the three Ficus species and their successive fractions was performed using high-performance liquid chromatography/electrospray ionization mass spectrometry. Their discrimination was carried out using the obtained spectral data applying chemometric unsupervised pattern-recognition techniques, namely, principal component analysis and hierarchical cluster analysis. In vitro hepatoprotective and antioxidant evaluation of the samples was performed using human hepatocellular carcinoma cells challenged by carbon tetrachloride (CCl₄).

Results: Altogether, 22 compounds belonging to polyphenolics, flavonoids, and furanocoumarins were identified in the three Ficus species. Apigenin is the most abundant compound in F. cordata while chlorogenic acid and psoralen were present in high percentages in F. ingens and F. palmata, respectively. Chemometric analyses showed that F. palmata and F. cordata are more closely related chemically to each other rather than F. ingens. The ethyl acetate fractions of all the examined species showed a marked hepatoprotective efficacy accounting for 54.78%, 55.46%, and 56.42% respectively. Chemometric analyses showed that Ficus palmata were chemically related to each other rather than F. ingens. Ficus species exhibited notable activities due to polyphenolics and furanocoumarins.

Key words: Antioxidant, chemometrics, chemotaxonomy, Ficus, hepatoprotective, phytochemical profiling

SUMMARY

- Ficus cordata, Ficus ingens, and Ficus palmata were analyzed using high-performance liquid chromatography/electrospray ionization mass spectrometry that revealed their richness with polyphenolics and furanocoumarins.
- Discrimination of the three species was performed using spectral data coupled with chemometrics that showed that F. palmata and F. cordata are chemically related to each other rather than F. ingens.
- In vitro hepatoprotective and antioxidant evaluation was performed using human hepatocellular carcinoma cells. The ethyl acetate fractions of all the examined species showed a marked hepatoprotective efficacy.

INTRODUCTION

Oxidative stress can be defined as an evident imbalance between the appearance of reactive oxygen species and the competence of the biological system to detoxify these hazardous intermediates effectively or to restore the explicit deterioration caused by them. Besides, it has recently been recognized as a predisposing factor to many fatal diseases as neurodegenerative disorders, including, Parkinson’s disease, Alzheimer’s...
disease as well as aging. Moreover, it is strongly correlated to liver diseases contributing to their ability to aggravate the inflammatory, metabolic, and proliferative hepatic changes that consequently leads to structural and functional anomalies in the liver.

Genus *Ficus* (family Moraceae) constitutes more than 850 species and about 2000 varieties, most of which are native to old-world tropics. Many of *Ficus* species are employed for many ornamental purposes whereas the fruits of others are edible. It has been widely implemented in African folk medicine for the treatment of many ailments such as convulsions and respiratory diseases. In addition, many members of *Ficus* were previously reported in both traditional Chinese medicine and Ayurveda medicine as a cure for many diseases such as diabetes, liver cirrhosis, and many inflammatory conditions.

Many biological activities, including antioxidant, hepatoprotective, antidiabetic, anti-inflammatory, antipyretic, antimicrobial, antimalarial, and hypotensive activities, have been ascribed to the genus *Ficus*. This could be probably due to the predominance of alkaloids, furanocoumarins, flavonoids, stilbenes, phenylpropanoids, lignans, chromones, and terpenoids in the genus. *Ficus cordata* exists in two separate areas in Africa, namely, the southwest of the continent and the northern subtropics. Its leaves are highly popular for possessing antimicrobial and hepatoprotective activity in addition to relief of ataxia as well as muscle tremor. However, *Ficus ingens*, the red-leaved fig, spreads in the tropical regions of Africa and southern Arabia. Recently, it has been reported to possess potent analgesic, anti-inflammatory as well as hepatoprotective effects that could be attributed to the presence of many active secondary metabolites. On the other hand, *Ficus palmata*, the wild fig, was adopted in the folk medicine for the relief of constipation, lung, and bladder ailments. It manifests potent antimicrobial, antioxidant, and nephroprotective efficacies owing to the presence of many phytoconstituents.

Nowadays, there is a revival of interest in herbal drugs due to the widespread belief that “green medicine” is relatively safer and more dependable than the costly synthetic drugs. Thus, adulteration of medicinal plants due to the presence of various species and varieties that are morphologically similar but biologically different constitutes a great obstacle threatening the future of herbal drug discovery. Therefore, chemometrics as an efficient discriminatory tool was adopted to differentiate between morphologically and chemotaxonomically related species.

In the foregoing study, we investigated comparatively the *in vitro* antioxidant and hepatoprotective activities of the methanol extracts prepared from the leaves of *F. cordata*, *F. ingens*, and *F. palmata* and their successive fractions. This was performed through the assessment of various hepatic markers as aspartate transaminase (AST) and alanine transaminase (ALT) in addition to different antioxidant parameters as reduced glutathione (GSH) and superoxide dismutase (SOD). In addition, profiling of the major secondary metabolites prevalent in these extracts and fractions was performed using high-performance liquid chromatography/electrospray ionization mass spectrometry (HPLC-ESI-MS) to correlate the activity with the predominant phytocomponents and to discriminate the three related *Ficus* species applying chemometric multivariate data analysis. The latter was performed for the first time using unsupervised pattern-recognition techniques using both hierarchical cluster analysis (HCA) and principal component analysis (PCA).

**MATERIALS AND METHODS**

**Plant material**

The aerial parts of *F. cordata* Thunb., *F. ingens* Miq., and *F. palmata* Forsk. (Moraceae) were collected from fully mature trees growing wild in the southern region of Saudi Arabia (Asir district, mainly Abha 900 Km away from Riyadh) in April 2010. The location is described by 18° 13′ 1″ N, 42° 30′ 19″ E, elevation 2400 m. The rainfall is estimated by 3–50 mm per annum and the average low and high temperatures are 12°C and 26°C, respectively. Samples from at least 19 trees for *F. cordata*, 13 trees for *F. palmata*, and 11 trees for *F. ingens* species were collected during the same vegetative phase to provide 1.0, 0.85, and 0.82 kg dried plant materials for the plants, respectively. They were kindly identified and authenticated morphologically by Dr. M. Atiqur Rahman, Plant Taxonomist, College of Pharmacy, King Saud University. Voucher specimens of *F. cordata* (#15133), *F. ingens* (#15187), and *F. palmata* (#15163) were deposited in the herbarium of the Pharmacognosy Department, College of Pharmacy, King Saud University.

**Chemicals and kits**

Media and all the required reagents for cell culture formation and maintenance comprising bovine serum albumin, Dulbecco’s Modified Eagle’s medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin solution were bought from Lonza (Basel, Switzerland). Ellman’s reagent and reduced GSH were purchased from Sigma-Aldrich (St. Louis, MO, USA), whereas silymarin, (Indena S.P.A, Milano, Italy) was obtained from Medical Union Pharmaceuticals Company (Cairo, Egypt). Kits for estimation of ALT, AST, and SOD activities were acquired from Biodiagnostics (Cairo, Egypt). Validation for all kits used was performed at the Department of Pharmacology, Ain Shams University (Cairo, Egypt), before experiments. Solvents for LC-MS analysis were kindly acquired from Sigma-Aldrich (Steinheim, Germany). All other utilized solvents in extraction and fractionation were of analytical and highest purity grades.

**Cell cultures**

Human hepatocellular carcinoma (HepG2) cell lines were obtained from the Egyptian Holding Company for Biological Products and Vaccines (VACSERA; Giza, Egypt) and then maintained in the tissue culture facility (Faculty of Pharmacy, Ain Shams University, Cairo, Egypt). They were kept in DMEM complete media (L-glutamine supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin). Cells were grown at 37°C in a humidified atmosphere of 5% CO₂. The cells were maintained as monolayer culture by serial subculturing. All the experiments were performed with cells in the logarithmic growth phase.

**Preparation of crude plant extracts**

The aerial parts of different *Ficus* species were air-dried and crushed into coarse powder to give 100 g each. Then, they were extracted with methanol (1.5 L) using a Soxhlet apparatus for 8 h. The obtained extracts were filtered and evaporated under vacuum at low temperature (45°C) till dryness using a rotary evaporator (Buchi, Switzerland) to give total methanol extract (F*T) for each. The methanol extracts were suspended in water and successively extracted with petroleum ether (F*P), ethyl acetate (F*E), and n-butanol (F*nB) to afford the corresponding subfractions in addition to the remaining aqueous fraction (F*A).

**High-performance liquid chromatography-mass spectrometry analysis**

All samples were prepared at a concentration of 40 µg in 1 mL methanol. The HPLC analysis was conducted on Agilent 1100 Series using Knauer column (250 mm × 2 mm, ID), prepacked with Eurospher 100–5 C18, with an integrated precolumn. For a standard LC-MS analysis, a solvent gradient started with acetonitrile: nanopure H₂O (10:90), adjusted
with 0.1% formic acid, and reached to 100% acetonitrile in 35 min was implemented. A Finnigan LCQ-DECA MS connected to a photodiode array detector with the standard flow cell (10 mm path length, 14 µL volume, and 40 bar maximum pressure) was used for MS analysis. The samples were dissolved in water/methanol mixtures and injected into HPLC/ESI-MS setup. ESI interface was used in both negative and positive ion modes under the following conditions: drying and nebulizing gas, N₂ capillary temperature, 250°C; spray voltage, 4.48 kV; capillary voltage, 39.6 V; tube lens voltage, 10.00 V; and full scan mode in mass range m/z 100–2000.

In vitro antioxidant and hepatoprotective activity assessment

The hepatoprotective activity of samples was tested in vitro at three different concentrations (25, 50, and 100 µg/mL) and compared to the standard hepatoprotective agent silymarin at the same concentrations. HepG2 monolayer cultures were pretreated with the assigned samples for 1 h. An aliquot of 40 mM carbon tetrachloride (CCl₄) in 0.05% dimethyl sulfoxide was added and incubation was continued for another 2 h. The supernatant medium and cell lysate were then collected and stored at −20°C until analysis. The positive control (silymarin) was assayed in cells maintained in culture medium and treated only with CCl₄ (40 mM) while the untreated control consisted of cells kept in phosphate-buffered saline. The levels of ALT and AST were assessed spectrophotometrically at 546 nm in the supernatant using commercially available kits according to the manufacturer’s instructions as previously reported (Biodiagnostics, Cairo, Egypt).[28]

The concentration of GSH and the activity of SOD were evaluated in cell lysates. GSH was determined by mixing equal volumes of the supernatant of a cell culture extract and 10% trichloroacetic acid–0.005 M ethylenediaminetetraacetic acid solution. This solution was then subjected to centrifugation at 6000 rpm for 15 min. To 0.5 mL of the resulting supernatant, 0.85 ml phosphate buffer (0.1 M, pH = 8) and 0.05 ml of 10 mM Ellman’s reagent 5,5'-dithiobis-(2-nitrobenzoic acid) were added, and the optical density of the stable yellow color developed by the reduction of Ellman’s reagent through the SH-group in GSH was measured colorimetrically at 412 nm.[24]

SOD activity was determined in the cell lysate through inhibition of pyrogallol autoxidation. Cytosolic fraction (20 µL) was added to a microcuvette containing 10 µL pyrogallol solution (10 mM dissolved in 10 mM HCl) and 1 ml Tris–HCl buffer (50 mM, pH = 8.2) containing 1 mM diethylene triamine pentaacetic acid. The change in absorbance per minute at 420 nm was recorded for 2 min.[23]

All the spectrophotometric measurements were carried out using a Shimadzu ultraviolet (UV)-1601 spectrophotometer (Kyoto, Japan).

Statistical and chemometric data analyses

Statistical analysis for biological assessment was expressed as means ± standard error of mean. Statistical comparison between different groups was performed using one-way analysis of variance, followed by Tukey–Kramer multiple comparison tests, to judge the difference between various groups. Statistical significance was accepted at P < 0.05. Graphs were plotted using GraphPad Prism version 5 software (GraphPad Software, Inc La Jolla, CA, USA). The data obtained from LC-MS for all samples and their replicates were transferred to an excel sheet by MS Excel® for multivariate analysis. The chemometric analysis of the data was performed using unsupervised pattern-recognition techniques applying both HCA and PCA. HCA was performed using Hierarchical Clustering Explorer 3.5 (Human-Computer Interaction Laboratory, University of Maryland, College Park, MD, USA), whereas PCA was performed by Unscrambler® 9.7 (CAMO SA, Oslo, Norway).

The data were subjected to preprocessing by mean centering of the raw data of all the samples before the analyses. The HCA was used to classify the sample into clusters applying the average group linkage method for cluster building in which the distance between clusters was calculated using Pearson’s correlation method. Meanwhile, in PCA, the constructed scatter score plots of the initial PCs are indicative of the similarity and variations among samples.[29]

RESULTS AND DISCUSSION

High-performance liquid chromatography/ electrospray ionization mass spectrometry profiling of the samples

HPLC-ESI-MS profiling of the major secondary metabolites in the aerial parts of three Ficus species reveals their richness in polyphenolics and furanocoumarins. Different polyphenolics and furanocoumarins were tentatively identified from the petroleum ether, ethyl acetate, butanol, and remaining aqueous fractions of F. cordata, F. ingens, and F. palmata by comparing their UV and LC-MS spectra (in both positive and negative ionization modes) with the published data [Tables 1-3].[27-46]

The results showed that polyphenolics comprising neochorogenic acid (2), cryptochlorogenic acid (5), and chlorogenic acid (7) were mostly abundant in the different fractions of F. ingens. Besides, several flavonoid glycosides such as acanthophorbin A (22) and B (30), myricitrin (23), infectorin (25), quercetin-3,4',3'-dimethylamine (31) together with a prenylated flavonoid, and 2'-O-methylarbutin (12) were identified in various fractions of all examined species. The identified furanocoumarins can be distributed into two major subclasses either glucosylated furanocoumarins or their aglycones. Glucosylated furanocoumarins include corylifolin-6-O-glucoside (11), n-Anethol A (13) and B (15), psoralenoside (17), and aviprin-3'-O-glucoside (28). The aglycones encompass corylifolin (19), psoralen acid (27), dihydropyrrolactic acid (20), aviprin (33) together with psoralen (32), and bergapten (34) in addition to 11-methoxyvincajamine (18), an alkaloid, that has been identified in the ethyl acetate fraction of F. cordata [Figure 1]. The obtained chromatograms were displayed in the supplementary materials.

Chemometric data analysis

The diversity of secondary metabolites, presents in various fractions of the three examined Ficus species as revealed by the HPLC-ESI-MS analyses, acts as a fundamental discriminatory tool through applying the unsupervised pattern-recognition techniques represented by PCA and HCA. PCA score plots showed the ability of all fractions to discriminate all the examined Ficus species without any overlapping by explaining 100% of the variance in the data, as shown in the first two PC1 and PC2.

In addition, the loading plots can partly express the influence of the different variables on the separation between classes. In both the petroleum ether and ethyl acetate fractions, acanthophorbin B and psoralen were the main active constituents discriminating F. ingens and F. palmata, respectively. Regarding F. cordata, bergapten and 11-methoxyvincajamine were the main characteristic components with the greatest influence on its segregation in the petroleum ether and ethyl acetate fractions, respectively.

Meanwhile, neochorogenic acid and infectorin were the main discriminating markers for F. ingens and F. palmata, respectively, in both the n-butanol and remaining aqueous fractions [Figure S6c and d]. Corylifolin-6-O-glucoside and aviprin represent the strongest variables for the segregation of F. cordata in n-butanol and the remaining aqueous fractions, respectively.
PCA score plot of all the tested samples [Figure 2] resulted in two orthogonal PCs, which explained about 70% of the variance in 180-dimensional space using only the first two components (the first PC accounts for 42% of the total variance followed by the second PC 28%). PCA plot could significantly discriminate *F. ingens* species in all the tested fractions (*F. ingens* petroleum ether fraction [FIP], *F. ingens* ethyl acetate fraction [FIE], *F. ingens* n-butanol fraction [FIB], and *F. ingens* remaining aqueous fraction [FIA]), where it was observed on the left side upward quadrant, whereas the right side of the plot, *Ficus palmata* petroleum ether fraction (FPF), and *Ficus palmata* ethyl acetate fraction (FPE) were located. However, *Ficus palmata* n-butanol fraction (FPB) and *Ficus palmata* remaining aqueous fraction (FPA) were clustered together with *Ficus cordata* petroleum ether fraction (FCP), *Ficus cordata* ethyl acetate fraction (FCF), *Ficus cordata* n-butanol fraction (FCB), and *Ficus cordata* remaining aqueous fraction (FCA). This PCA pattern indicated the chemical closeness of *F. palmata* to *F. cordata*.

Moreover, HCA was performed by applying the average group linkage method for cluster building, and the distance between clusters is computed by Pearson's correlation method. The obtained dendrogram showed four main clusters, revealing the close distance of *F. palmata* to *F. cordata* that confirmed both species are more closely related chemically to each other rather than *F. ingens* [Figure 3].

### Antioxidant and hepatoprotective assessment

The evaluation of the antioxidant and hepatoprotective activity was carried out *in vitro* using the HepG2 cells, where CCl₄ was chosen as a hepatotoxic agent to induce the oxidative stress in cell lines. In general, a marked elevation in the serum level of ALT and AST enzymes (*P < 0.05*) was noticed in CCl₄-treated cells estimated by 65.90 and 49.38%, respectively, comparable to normal cells.

The ethyl acetate fraction of all the examined *Ficus* species showed significant concentration-dependent amelioration of CCl₄-induced damage as evidenced from values of ALT and AST. It is worthy to mention that FCE, FIE, and FPE produced 54.78%, 55.46%, and 56.42% suppression in serum level of ALT, respectively, at 100 μg/ml. Moreover, it was indicated that FCE, FIE, and FPE produced 54.78%, 55.46%, and 56.42% suppression in serum level of AST, respectively, at 100 μg/ml.
37.27% and 51.99% decrease in ALT and AST, respectively, at 100 µg/mL [Tables 4-6]. This was subsequently followed by the n-butanol fraction that also showed a pronounced decline in ALT and AST levels with concomitant improvement of CCl₄-induced damage. FCB, FIB, and FPB
treated cells showed 33.81%, 48.30%, and 50.60% reduction in ALT and 52.80%, 52.69%, and 55.94% decline in AST, respectively, at a dose of 100 µg/mL. Moreover, at a dose of at 100 µg/ml, FCT, FCP, and FCA displayed moderate amelioration in HepG2 cells damage induced by CCl₄, showing 33.93%, 24.09%, and 29.89% decline in ALT and 34.40%, 48.00%, and 46.94% lowering in AST serum levels, respectively. On the other hand, FIT, FIP, and FIA showed 25.35%, 29.70%, and 37.26% decrease in ALT as well as 34.58%, 42.26%, and 52.13% reduction in AST, respectively. However, FPT, FPP, and FPA were nonsignificant from the normal control as well as the silymarin-treated cells revealing 39.21%, 32.16%, and 42.58% fall in ALT leakage from hepatic cells as well as 48.63%, 48.09%, and 57.71% lowering in AST levels [Figure 4].

Regarding pretreatment of HepG2 cells with the n-butanol fractions, namely, FCB, FIB, and FPB resulted in a pronounced elevation in the antioxidant parameters showing 39.21%, 32.16%, and 42.58% fall in ALT leakage from hepatic cells as well as 48.63%, 48.09%, and 57.71% lowering in AST levels [Figure 4].

To sum up, the ethyl acetate fraction followed by the n-butanol fractions of all the examined Ficus species showed significant antioxidant and hepatoprotective effects as evidenced by the amelioration of AST and ALT as well as replenishing of GSH and SOD in the treated cells [Figure 4]. These could be partly explained in view of the presence of plenty of phytoconstituents as the ethyl acetate followed by n-butanol exhibited the best extractive power of the polyphenolics and furanocoumarins reflected by the higher number of peaks identified in the respective HPLC chromatograms. Polyphenolics and furanocoumarins act as free...
Table 4: Antioxidant and hepatoprotective activities of the various fractions of Ficus cordata aerial part (alanine transaminase, aspartate transaminase, reduced glutathione, and superoxide dismutase)

| Groups                      | ALT (U/mL)     | AST (U/mL)     | GSH (nmol/mg protein) | SOD (U/mL)     |
|-----------------------------|----------------|----------------|-----------------------|----------------|
| Control                     | 62.44±2.48     | 55.26±3.81     | 16.19±0.78*           | 358.71±13.74*  |
| CCl<sub>4</sub>             | 103.59±1.46    | 109.17±2.13    | 7.66±0.47             | 0.00±0.01     |
| Sil (100 µg/mL)             | 64.98±0.80*    | 52.41±1.17*    | 16.8±0.08*            | 308.93±3.64*  |
| Sil (50 µg/mL)              | 67.90±0.26*    | 56.67±0.38*    | 16.59±0.08*           | 275.00±7.93*  |
| Sil (25 µg/mL)              | 73.29±0.69*    | 64.53±1.02*    | 15.08±0.06*           | 212.20±0.48*  |
| F. cordata methanol extract (µg/mL) |                |                |                       |               |
| 100                         | 68.44±4.64*    | 71.62±4.29*    | 13.47±0.11*           | 221.43±3.56*  |
| 50                          | 72.92±1.54*    | 79.79±2.21*    | 13.11±0.10*           | 144.64±11.36* |
| 25                          | 83.07±3.00*    | 82.46±2.27*    | 12.59±0.23*           | 105.26±0.33*  |
| F. cordata ether acetate fraction (µg/mL) |                |                |                       |               |
| 100                         | 78.63±2.87*    | 56.76±1.63*    | 12.60±0.05*           | 226.04±30.62* |
| 50                          | 79.59±1.46*    | 59.00±0.80*    | 12.34±0.07*           | 151.55±11.02* |
| 25                          | 81.35±1.49*    | 61.24±1.29*    | 12.04±1.00*           | 105.26±0.33*  |
| F. cordata n-butanol fraction (µg/mL) |                |                |                       |               |
| 100                         | 47.88±1.65*    | 47.14±0.81*    | 16.18±0.23*           | 293.67±28.35* |
| 50                          | 60.24±0.55*    | 48.62±0.37*    | 15.17±0.50*           | 251.65±11.08* |
| 25                          | 66.02±0.68*    | 51.81±0.95*    | 14.89±0.34*           | 197.09±0.93*  |
| F. cordata aqueous fraction (µg/mL) |                |                |                       |               |
| 100                         | 68.56±1.43*    | 51.32±1.27*    | 16.32±0.24*           | 256.25±22.17* |
| 50                          | 73.22±0.76*    | 53.64±1.35*    | 15.61±0.71*           | 188.14±16.00* |
| 25                          | 75.09±1.21*    | 62.61±2.45*    | 14.69±0.05*           | 170.76±0.13*  |

*Significantly different from CCl<sub>4</sub> at P<0.05; **Significantly different from Sil (100 µg/mL) at P<0.05; ***Significantly different from Sil (50 µg/mL) at P<0.05. Data are measured in triplicates (n=3) and presented as means±SEM. ALT: Alanine transaminase; AST: Aspartate transaminase; GSH: Glutathione; Sil: Silymarin; F. cordata: Ficus cordata; SEM: Standard error of mean; FCB: F. cordata n-butanol fraction

Table 5: Antioxidant and hepatoprotective activities of the various fractions of Ficus ingens aerial part (alanine transaminase, aspartate transaminase, reduced glutathione, and superoxide dismutase)

| Groups                      | ALT (U/mL)     | AST (U/mL)     | GSH (nmol/mg protein) | SOD (U/mL)     |
|-----------------------------|----------------|----------------|-----------------------|----------------|
| Control                     | 62.44±2.48     | 55.26±3.81     | 16.19±0.78*           | 358.71±13.74*  |
| CCl<sub>4</sub>             | 103.59±1.46    | 109.17±2.13    | 7.66±0.47             | 0.00±0.01     |
| Sil (100 µg/mL)             | 64.98±0.80*    | 52.41±1.17*    | 16.8±0.08*            | 308.93±3.64*  |
| Sil (50 µg/mL)              | 67.90±0.26*    | 56.67±0.38*    | 16.59±0.08*           | 275.00±7.93*  |
| Sil (25 µg/mL)              | 73.29±0.69*    | 64.53±1.02*    | 15.08±0.06*           | 212.20±0.48*  |
| F. ingens methanol extract (µg/mL) |                |                |                       |               |
| 100                         | 77.33±2.40*    | 71.42±3.53*    | 13.12±1.77*           | 294.64±0.34*  |
| 50                          | 85.59±1.93*    | 83.66±2.83*    | 12.67±1.66*           | 217.86±0.41*  |
| 25                          | 90.07±1.60*    | 90.14±2.35*    | 11.88±1.70*           | 169.64±0.73*  |
| F. ingens ether acetate fraction (µg/mL) |                |                |                       |               |
| 100                         | 72.82±1.55*    | 63.03±2.25*    | 11.42±0.20*           | 144.64±16.67* |
| 50                          | 76.26±1.80*    | 68.01±2.62*    | 10.54±1.29*           | 082.14±0.73*  |
| 25                          | 80.77±1.20*    | 74.56±1.74*    | 09.27±0.07*           | 071.43±0.48*  |
| F. ingens n-butanol fraction (µg/mL) |                |                |                       |               |
| 100                         | 46.14±0.61*    | 50.04±1.42*    | 18.68±0.10*           | 320.94±0.08*  |
| 50                          | 56.31±2.02*    | 52.17±1.17*    | 16.64±0.18*           | 272.91±0.65*  |
| 25                          | 68.36±1.17*    | 55.94±2.03*    | 15.27±0.04*           | 176.04±0.98*  |
| F. ingens aqueous fraction (µg/mL) |                |                |                       |               |
| 100                         | 53.56±0.48*    | 51.65±0.97*    | 19.29±0.80*           | 291.67±0.80*  |
| 50                          | 59.75±0.15*    | 53.58±2.88*    | 17.02±0.70*           | 168.75±0.40*  |
| 25                          | 69.67±1.00*    | 58.31±1.43*    | 15.13±0.08*           | 156.25±0.72*  |

*Significantly different from CCl<sub>4</sub> at P<0.05; **Significantly different from Sil (100 µg/mL) at P<0.05; ***Significantly different from Sil (50 µg/mL) at P<0.05. Data are measured in triplicates (n=3) and presented as means±SEM. ALT: Alanine transaminase; AST: Aspartate transaminase; GSH: Glutathione; Sil: Silymarin; F. ingens: Ficus ingens; FIB: F. ingens n-butanol fraction
radical scavengers mediated by their ability to bind transition metals, iron and copper by adjacent –OH groups, or other chelating structures and thus inhibiting the free radical chain reactions and explaining their antioxidant potential.\(^{[48,49]}\)

**Table 6:** Antioxidant and hepatoprotective activities of the various fractions of *F. palmata* aerial part (alanine transaminase, aspartate transaminase, reduced glutathione, and superoxide dismutase)

| Groups                          | ALT (U/mL) | AST (U/mL) | GSH (nmol/mg protein) | SOD (U/mL) |
|--------------------------------|------------|------------|-----------------------|------------|
| Control                        | 62.44±2.48* | 55.26±3.81* | 16.19±0.78*           | 358.71±13.74* |
| CCl                             | 103.59±1.46 | 109.17±2.13 | 07.66±0.47            | 050.00±01.82  |
| Sil (100 μg/mL)                 | 64.98±0.80* | 52.41±1.17* | 16.86±0.08*           | 308.93±03.64* |
| Sil (50 μg/mL)                  | 67.90±0.26* | 56.67±0.38* | 16.59±0.08*           | 275.00±07.93* |
| Sil (25 μg/mL)                  | 73.29±0.69* | 64.53±1.02* | 15.08±0.06*           | 212.20±04.81* |
| *F. palmata* methanol extract (μg/mL) |           |            |                       |            |
| 100                            | 62.97±1.40* | 56.08±2.15* | 16.98±0.47*           | 348.21±06.30* |
| 50                             | 69.25±1.32* | 65.72±2.03* | 16.39±0.19*           | 294.64±16.67* |
| 25                             | 74.49±1.41* | 73.72±2.17* | 14.44±0.19*           | 239.29±18.19* |
| *F. palmata* pet. ether fraction (μg/mL) |           |            |                       |            |
| 100                            | 70.27±1.12* | 56.67±1.60* | 13.51±0.29*           | 267.86±03.15* |
| 50                             | 70.63±0.77* | 57.19±1.10* | 12.71±0.10*           | 219.64±03.15* |
| 25                             | 72.82±0.53* | 60.29±0.76* | 12.22±0.23*           | 101.79±05.46* |
| *F. palmata* ethyl acetate fraction (μg/mL) |           |            |                       |            |
| 100                            | 45.14±1.04* | 46.88±0.45* | 16.92±0.74*           | 352.14±07.51* |
| 50                             | 55.22±1.85* | 47.51±0.23* | 16.49±0.36*           | 295.77±09.08* |
| 25                             | 62.48±1.82* | 49.91±1.00* | 15.76±0.20*           | 249.21±10.33* |
| *F. palmata* n-butanol fraction (μg/mL) |           |            |                       |            |
| 100                            | 54.17±1.37* | 48.10±0.11* | 17.89±0.05*           | 325.25±05.87* |
| 50                             | 62.38±0.45* | 51.01±0.65* | 17.11±0.19*           | 268.00±11.69* |
| 25                             | 67.06±1.31* | 54.69±0.53* | 16.87±0.33*           | 221.65±07.13* |
| *F. palmata* aqueous fraction (μg/mL) |           |            |                       |            |
| 100                            | 59.17±1.36* | 46.17±0.22* | 16.53±0.14*           | 298.34±07.95* |
| 50                             | 61.01±0.94* | 49.86±2.62* | 17.86±0.16*           | 225.09±04.59* |
| 25                             | 64.45±1.76* | 53.45±1.40* | 17.38±0.11*           | 179.09±07.56* |

*Significantly different from CCl\(_4\) at P<0.05; **Significantly different from Sil (100 μg/mL) at P<0.05; ***Significantly different from Sil (50 μg/mL) at P<0.05; ****Significantly different from Sil (25 μg/mL) at P<0.05. Data are measured in triplicates (n=3) and presented as means±SEM. ALT: Alanine transaminase; AST: Aspartate transaminase; SOD: Superoxide dismutase; GSH: Glutathione; Sil: Silymarin; SEM: Standard error of mean; *F. palmate*: *Ficus palmate*; FPE: *F. palmata* ethyl acetate fraction
CONCLUSION

The methanol extracts as well as the successive fractions of the aerial parts of *F. cordata*, *F. ingens*, and *F. palmata* are rich sources of polyphenolics and furanocoumarins as tentatively identified by the HPLC-ESI-MS. These secondary metabolites serve as powerful discriminatory tools for these species through applying chemometrics multivariate analysis techniques particularly unsupervised pattern-recognition techniques, namely, PCA and HCA. In addition, polyphenolics and furanocoumarins greatly contributed to the hepatoprotective activity of the tested samples and their tendency to combat oxidative stress. This will shed a light on the potential use of the various *Ficus* species as promising hepatoprotective agents. However, isolation of the secondary metabolites with subsequent in vivo biological assessment is required to ascertain the claimed results.

Acknowledgement

The authors would like to acknowledge Dr. Eckhard Roth (BIO-MAR Company) and Prof. Dr. Peter Proksch, (Heinrich-Heine Universität, Düsseldorf, Germany) for conducting LC-MS analyses using their facilities.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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