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Quantitative Analysis of Total Phenolic and Total Flavonoid Constituents of some *Ficus* species

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Abstract

The study was designed to compare the total phenolic and flavonoid contents of the total methanolic extracts of seven *Ficus* species (*F. bengalensis, F. decora, F. hawaii, F. virens, F. platyphylla, F. retusa, and F. cycomorous*) cultivated in Egypt. Leaves of the different *Ficus* species were each separately extracted with methanol, and dried under vacuum to give a syrupy consistency. All dried extracts showed a wide variation of total phenolic contents when tested by Folin–Ciocalteu method ranging from 2.507±0.715 to 7.9744±0.565mg gallic acid equivalent/g dried extract. Total flavonoid contents were evaluated using modified Aluminum chloride colourimetric method. The results were ranged from 1.8571±0.658 to 12.4643±0.366 mg rutin equivalent/g dried extract. Results of various assays were analyzed statistically by applying appropriate statistical methods.

Key words
Total phenolic, Total flavonoid, Ficus, Moraceae

1. Introduction

Family Moraceae (Mulberry) is one of the largest families among angiosperms, comprising 73 genera and 1100 species worldwide, of trees, shrubs, climbers and herbs with a milky latex (Berg 2001). Several members of the genus *Ficus (F. Moraceae)* have a wide variety of medical uses all over the world (Kone, Atindehou et al. 2004) (Lansky, Paavilainen et al. 2008). Phytochemical investigations of some *Ficus* species revealed that phenolic compounds constitute the majority of components (Li, Bu et al. 2006) (Yu, Haley et al. 2002). Also, some studies reported the antioxidant activity of some *Ficus* species which had been attributed to their phenolic contents (Al-Fatimi, Wurster et al. 2007) (Manian, Anusuya et al. 2008) (Shukla, Gupta et al. 2004). Several *Ficus* species are indigenous to Egypt, such as *F. benghalensis, F. cycomorous, F. retusa, F. platyphtlla, F. virens, F. decora,* and *F. hawaii*. (El-Hadidi and Boulos 1988). Some phenolic compounds were isolated from *Ficus* leaves, namely, furanocoumarins like psoralen and bergapten, flavonoids like 3-O-rutinoside and phenolic acids like ferulic acid, 3- O-caffeoylquinic acid and 5-O-caffeoylquinic acid (Oliveira, Baptista et al. 2012). Some *Ficus* species also showed the presence of different classes of flavonoids (Lansky, Paavilainen et al. 2008). Flavonoids have important medicinal uses such as anti-cancer, anti-allergic and anti-inflammatory activities (Crozier, Yokota et al. 2006).

Previously, the quantitative analysis of the total phenolic and flavonoid contents of the n-butaol and ethyl acetate soluble fractions of some Egyptian *Ficus* species including *F. cycomorous, F. retusa and F. decora* was reported (Abdel-Hameed 2009).

This study was aimed to investigate and compare the total phenolic and flavonoid contents of leaf methanolic extracts obtained from seven *Ficus* species cultivated in Egypt.

2. Materials, Apparatus and Techniques:

2.1. Solvents and chemicals

MeOH of HPLC grade (Merck Co., Darmstadt, Germany), rutin, gallic acid, aluminum chloride and Folin-Ciocalteu (Sigma Co., St. Louis, MO, USA). Sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH) and sodium nitrite ($NaNO_2$) (Wako Co., Osaka, Japan).

2.2. Instruments

UV-Visible Spectrophotometer (SPECTRONIC ® GENESYS 2PC UV, USA) used for spectrophotometric analysis.

2.3. Preparation of Extracts

Fresh leaves of *Ficus species (F. bengalensis, F. platyphylla, F. hawaii, F. decora, F. cycomorus, F. virens, and F. retusa)* were collected from the Experimental Station of Ornamental Plants, Faculty of Agriculture, Minia University. Each species was separately dried in the shade at room temperature and reduced to coarse powder, and then all were separately extracted by maceration with methanol. Each of the methanolic extracts was concentrated under vacuum to a syrupy residue.

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2.4. Evaluation of total phenolic contents

The total phenolic content of each plant extract was determined by Folin–Ciocalteu method (Singleton and Rossi 1965). For preparation of the calibration curve, 50 µl aliquots of 0.25, 0.50, 1 and 2 mg/ml methanolic gallic acid solutions were mixed with 50 µl Folin–Ciocalteu reagent (2N), 300µl (20 % anhydrous sodium carbonate) and 3.5 ml deionized water. The absorption was measured after 30 min at λ_{max} 728 nm compared to a blank of absolute methanol using UV-spectrophotometer.

The methanolic extracts of each *Ficus* species 50 µl (10 mg/ml) was separately treated as mentioned above, and after 30 min the absorption was measured at λ_{max} 728 nm. All determinations were performed in triplicates and the total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram dried extract.

2.5. Evaluation of total flavonoid contents

The total flavonoids content of the methanol extracts of each species was determined using a modified colourimetric method (Zhishen, Mengcheng et al. 1999). For preparation of a calibration curve, 2ml aliquots of 0.2, 0.3, 0.4 and 0.5 mg/ml methanolic rutin solutions, were each mixed with 300µl $NaNO_2$ (5%) and left for 6 min, then 300 µl of (10 % $AlCl_3$) was added and allowed to stand for another 6 min followed by 1 ml of NaOH (4 %), then deionized water was added immediately to bring the final volume to 6 ml, where the mixture was thoroughly mixed and allowed to stand for 15 min. The absorption was measured at λ_{max} 510 nm. For each concentration, three determinations were carried out, then the absorbance for each was plotted versus its concentration and the calibration curve was drawn.

A 25 mg of each methanol extract of *Ficus* sp. was dissolved in 10 mL of methanol to obtain the working samples for this study. A 2ml methanolic plant extract (2.5 mg/ml) was mixed with the same reagents as described above, and after 15 min the absorption was measured at λ_{max} 510 nm. All determinations were performed in triplicates.

2.6. Statistical analysis

Results were expressed as mean ± SEM, and analyzed by the one-way analysis of variance (ANOVA) test using the Graph Pad Prism 6 software (Version 6.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

3. Results and Discussion

Folin–Ciocalteu's assay was used for measuring the total phenolics while evaluation of the total flavonoids achieved by aluminium chloride method. The Folin–Ciocalteu's assay is a common methods used to evaluate the total phenolic contents of medicinal plants (Roginsky and Lissi 2005). Reaction of Phenolic with Folin–Ciocalteu reagent (FCR) occurs in alkaline medium (using anhydrous sodium carbonate 20%). As a result of this reaction, Dissociation of a phenolic proton occurs to produce a phenolate anion, which is the reason behind reducing FCR, where the molybdate in testing system is reduced forming molybdenum oxide which is the source of blue colour. This blue colour has maximum absorption at λ_{max} 728 nm. The total quantity of phenolic compounds present in the samples is proportional to intensity of blue colour produced (Abdel-Hameed 2009).

Analysis of the total phenolic contents of all extracts revealed that the total methanolic extracts of *Ficus cycomorous*, *Ficus decora* and *Ficus bengalensis* showed the highest total phenolic contents, of values 7.97, 7.60 and 5.88 mg/ g gallic acid equivalent, respectively. *Ficus platyphylla*, *Ficus hawaii* and *Ficus retusa* showed moderate total phenolic contents which were 4.10, 3.91 and 3.77 mg/ g gallic acid equivalent, respectively. However, *Ficus virens* showed the lowest phenolic content which was 2.50 mg/ g gallic acid equivalent. (**Table 1**, **Figure 1**). Determining the total flavonoid content was done by aluminium chloride colorimetric method which is based on the formation of a stable complex between the keto and hydroxyl groups of flavones and flavonoids with aluminium chloride. Furthermore, determination of total flavonoid contents utilized that the total methanolic extract of *Ficus decora* showed the highest total flavonoid content which was 12.46 mg/g rutin equivalent. The extracts of *Ficus bengalensis*, *Ficus cycomorous*, *Ficus hawaii* and *Ficus retusa* showed moderate total flavonoid content which were 5.88, 4.53, 3.32, and 3.20 mg/g rutin equivalent, respectively.

Concomitantly, the methanolic extracts of *Ficus platyphylla* and *Ficus virens* showed the lowest flavonoid content of 2.01 and 1.85 mg/g rutin equivalent, respectively (**Table 2**, **Figure 2**).

Table 1: Total phenolic content of the methanolic extracts of seven Ficus species

Plant extracts	Total phenolics (mg gallic acid equivalent/g dried extract)
Ficus cycomorous	7.9744±0.565
Ficus decora	7.6000±0.69
Ficus bengalensis	5.8800±0.49
Ficus platyphylla	4.1003±0.376
Ficus hawaii	3.9134±0.49
Ficus retusa	3.7750±0.98
Ficus virens	2.5070±0.715

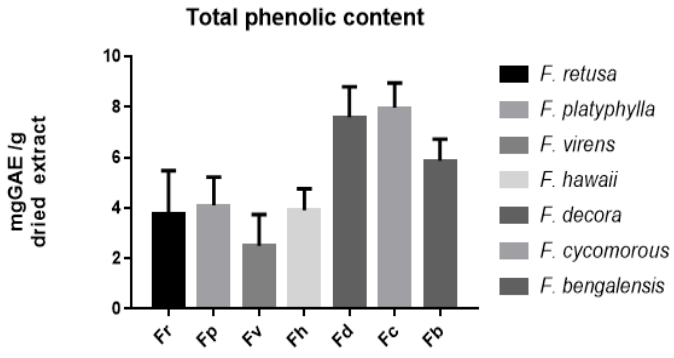

Figure 1: Total phenolic content of the methanolic extracts of seven Ficus species

Table 2: Total flavonoid content of the methanolic extracts of seven Ficus species

Plant extracts	Total flavonoid (mg rutin equivalent/g dried extract)
Ficus decora	12.4643±0.366
Ficus bengalensis	5.8800±0.548
Ficus cycomorous	4.5350±0.508
Ficus hawaii	3.32140±0.513
Ficus retusa	3.2023±1.096
Ficus platyphylla	2.0110±0.710
Ficus virens	1.8571±0.658

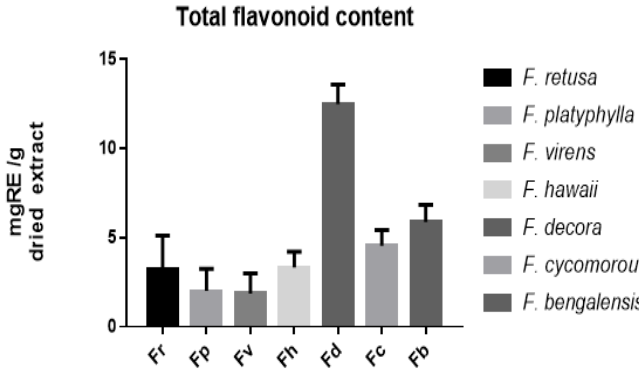

Figure 2: Total flavonoid content of the methanolic extracts of seven Ficus species

4. Conclusion

The pre-mentioned results provide evidence that the seven investigated *Ficus* species cultivated in Egypt contain phenolic compounds and flavonoid derivatives which may provide a wide variety of biological activities. This provoked us to further investigate their antimicrobial and antioxidant activity, which may result in discovering new antibiotic drugs from natural sources.

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