

## Study of the Antioxidant and Anti-Inflammatory Potential of the Aerial Parts of *Ficus nitida* L. (Moraceae) and its Phytochemical Composition

Khaled Rashed,<sup>1\*</sup> Ching-Shu Lai,<sup>2</sup> Min-Hsiung Pan,<sup>3,4</sup> Chistiane Feitosa<sup>5</sup>

<sup>1</sup> Pharmacognosy Department, National Research Centre, 33 El-Bohouth St., Dokki, Giza, Egypt.

<sup>2</sup> Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung, Taiwan.

<sup>3</sup> Hubei Key Laboratory of Economic Forest Germplasm Improvement and Resources Comprehensive Utilization, College of Life Science, Huanggang Normal University, Hubei, China.

<sup>4</sup> Institute of Food Science and Technology, National Taiwan University, Taipei 10617, Taiwan.

<sup>5</sup> Federal University of Piaui, Laboratory of Natural Products, Chemistry Department, Ininga, Teresina-Pi, Brazil.

### ABSTRACT

The aim of this investigation was to evaluate the antioxidant and anti-inflammatory activities of a methanolic extract of the aerial parts of *Ficus nitida*, and to investigate the main phytoconstituents present in the extract. The extract was tested for antioxidant activity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), and for anti-inflammatory potential by using a nitrite assay. The extract was also screened for phytochemicals by different chemical tests. The results showed that the *Ficus nitida* extract had significant antioxidant and anti-inflammatory effects that were concentration-dependent. Phytochemical analysis of the methanol extract proved the presence of alkaloids and/or nitrogenous compounds, carbohydrates and/or glycosides, flavonoids, tannins, and triterpenes and/or sterols. These results indicated that a methanolic extract of *Ficus nitida* aerial parts may act as a potential anti-inflammatory and antioxidant.

**Keywords:** *Ficus nitida*, Aerial parts, Antioxidant, Anti-inflammatory activity, Phytoconstituents.

### 1. INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants.<sup>1</sup> It is a protective attempt by the organism to remove the injurious stimuli as well as to initiate the healing process of the tissue.<sup>2</sup> It is characterized by five cardinal signs: Pharmacologists throughout the world have been focused on finding safer and more potent anti-inflammatory drugs. In addition, people have been turning back to natural products with the hope of finding treatments with which they feel more comfortable.

The genus *Ficus* is made up of about 1,000 species from pantropical and subtropical origins.<sup>3</sup> *Ficus nitida* L.

(Moraceae) is a widely cultivated, ornamental tree in Egypt. The genus *Ficus* (Moraceae) includes some 750 species of woody plants occurring in most tropical and subtropical forests throughout the world. *Ficus nitida* L. (Moraceae) is a widely cultivated, ornamental tree in Egypt. Many *Ficus* species are commonly used in traditional medicine to cure various diseases. They have long been used in folk medicine as astringents, carminatives, stomachics, hypotensives, anthelmintics and antidiarrhetics.<sup>4</sup> This study was undertaken in order to investigate the antioxidant and anti-inflammatory activities of *Ficus nitida* L., and to investigate its main phytoconstituents.

### Materials and methods

#### Plant identification and collection

*Ficus nitida* aerial parts were collected from Al-Zohriya Garden, Giza, Egypt in May 2011. The plant was

\* khalednabih2015@yahoo.co.uk

Received on 18/10/2017 and Accepted for Publication on 14/1/2018.

identified by Dr. Mohammed El-Gebaly of the Department of Botany, National Research Centre (NRC), and by Mrs. Tereza Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and Director of Orman Botanical Garden, Giza, Egypt. A voucher specimen was deposited in the herbarium of Al-Zohriya Garden, Giza, Egypt.

### Extraction and isolation of the phytochemical compounds

Powdered aerial parts from *Ficus nitida* (200 g) were extracted with methanol until exhaustion. The dried extract totaled 8.5 g. A phytochemical investigation was conducted according to Yadav and Agarwala.<sup>5</sup> The methanolic extract of *F. nitida* was dissolved in dimethyl sulfoxide (DMSO), and control cells were treated with 0.05% DMSO as a vehicle.

### Antioxidant Activity

#### 1.1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was evaluated according to the method of Lo et al.<sup>6</sup> Briefly, different concentrations of the *F. nitida* extract and the control solution were mixed in 1 mL of freshly prepared 0.1 mM DPPH solution (in 60% absolute alcohol). The mixtures were shaken vigorously and then incubated at room temperature in the dark for 30 min. Absorbance of the mixture at 517 nm was measured immediately with an enzyme-linked immunosorbent assay (ELISA) plate reader. DPPH scavenging activity was calculated according to the following equation:

$$\text{DPPH scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

### Anti-Inflammatory Activity

#### Nitrite Assay

Murine macrophage RAW264.7 cells were treated with either various concentrations of *F. nitida* extract plus lipopolysaccharide (LPS) 100 ng/mL, or with LPS only for 24 h. The nitrite concentration in the culture medium was measured as an indicator of nitrite (NO) production,

according to the Griess reaction. After centrifugation at 1000 g for 20 min, 100 µL of each supernatant medium were mixed with the same volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride in water). Absorbance of the mixture at 550 nm was measured with an ELISA plate reader.

### Results and Discussion

The antioxidant activity of *F. nitida* extracts was measured by using the stable radical DPPH, which is widely used to evaluate the potential for plant extracts to be free radical scavengers.<sup>7</sup> As presented in Table 1, the scavenging ability of *F. nitida* extract was concentration-dependent. In this study, the percent of scavenging activity reached a maximum (70%) at 100 µg/mL of *F. nitida* extract. The radical scavenging capacity of the *F. nitida* extract may be related to the phenolic hydroxyl groups of its phytochemicals (Table 3).

**Table 1. Antioxidant activity of *F. nitida* extract at different concentrations as indicated by DPPH free radical scavenging activity (%)<sup>a</sup>**

	Sample (2) <sup>b</sup>
20 µg /mL	28.3 ± 2.1
40 µg /mL	45.9 ± 3.9
60 µg /mL	59.7 ± 0.2
80 µg /mL	73.6 ± 5.6
100 µg /mL	76.0 ± 1.9

<sup>a</sup> DPPH: 1,1-diphenyl-2-picrylhydrazyl.

<sup>b</sup> Values are expressed as means ± SE; tests at each concentration were performed in triplicate.

To investigate the anti-inflammatory effects of the methanolic extracts of *F. nitida*, the levels of nitrite in culture media of RAW264.7 cells was examined. As shown in Table 2, the level of nitrite was increased in the LPS-treated group. On the other hand, cells co-treated with various concentrations of methanolic *F. nitida* extract dose-dependently suppressed LPS-induced nitrite production.

**Table 2. Anti-inflammatory activity of *F. nitida* extract at different concentrations as indicated by suppression of LPS-induced nitrite production<sup>a</sup>**

	Nitrite level ( $\mu\text{M}$ ) <sup>b</sup>		
	Sample (2)		
Control	1.4 $\pm$ 0.1		
LPS	13.0 $\pm$ 0.1		
20 $\mu\text{g}/\text{mL}$ +LPS	9.9 $\pm$ 1.5		
40 $\mu\text{g}/\text{mL}$ +LPS	8.4 $\pm$ 0.3		
60 $\mu\text{g}/\text{mL}$ +LPS	6.9 $\pm$ 0.5		
80 $\mu\text{g}/\text{mL}$ +LPS	5.0 $\pm$ 0.0		
100 $\mu\text{g}/\text{mL}$ +LPS	3.8 $\pm$ 1.0		

<sup>a</sup> LPS: lipopolysaccharide (100ng/mL). <sup>b</sup> Values are expressed as means  $\pm$  SE; tests at each concentration, for the control and for the LPS-only solution were performed in triplicate.

**Table 3. Phytochemical analysis of the methanolic extract of the aerial parts of *F. nitida***

Constituent	Presence of constituent in <i>F. nitida</i> extract <sup>a</sup>
Triterpenes and /or sterols	+
Carbohydrates and/or glycosides	+
Flavonoids	+
Coumarins	-
Alkaloids and/or nitrogenous compounds	+
Tannins	+
Saponins	-

<sup>a</sup>+: presence of constituents; -: absence of constituents

The phytoconstituents of the *F. nitida* extract were identified as triterpenes and/or sterols, carbohydrates and/or glycosides, flavonoids, alkaloids and/or nitrogenous compounds, and tannins (Table 3). It has previously been reported that naturally occurring flavonoids regulate NO production through a variety of mechanisms and are known as potential anti-inflammatory agents.<sup>8</sup> Triterpenes isolated from mushroom *Ganoderma lucidum* have also been found to inhibit LPS-induced NO production and iNOS protein expression in macrophages.<sup>9</sup> In addition, alkaloids isolated from several plants

including *Chelidonium majus*, *Liparis nervosa* and *Tripterygium wilfordii* were found to inhibit LPS-induced NO production.<sup>10-12</sup>

### Conclusion

Thus, we suggested the potential of methanol extract of *F. nitida* on suppression of NO production was contribute to those phytochemicals. However, identification of the individual phytochemicals in *F. nitida* extract and their contents may require further study.

### REFERENCES

- (1) Ferrero-Miliani L., Nielsen O.H., Anderse P.S. and Girardin S.E. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clin. Exp. Immunol.* 2007; 23:147-151.
- (2) Abass AK, Fausto N, Kumar V. Acute and chronic inflammation, In: Robbins and Cotran pathologic basis

- of disease, 7<sup>th</sup> Edn. Elsevier 2004; 47-87.
- (3) Wagner WL, Herbst DR, Sohmer SH. Manual of the Flowering Plants of Hawaii. Vol 2, University of Hawaii and Bishop Museum Press, Honolulu, 1999.
- (4) Khare, C.P. Encyclopedia of Indian Medicinal Plants Springer publication; 2004; 216-217.
- (5) Yadav, R.N.S., Agarwala, M. Phytochemical analysis of some medicinal plants. *Journal of Phytol.* 2011; 3, 10-14.
- (6) Lo, A.H, Liang, Y.C, Lin-Shiau, S.Y., Ho, C.T., Lin, J.K. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor- $\kappa$ B in mouse macrophages. *Carcinogen.* 2002; 23 (6): 983-91.
- (7) Krystyna, P., Anna, P. Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Anal. Methods* 2013; 5: 4288-95.
- (8) Kim, A.R., Cho, J.Y, Yani, Z., Jae, S.C, Chung H.Y. Flavonoids differentially modulate nitric oxide production pathways in lipopolysaccharide-activated RAW264.7 cells; Anti-inflammatory activity of natural dietary flavonoids. *Arch Pharm Res* 2005; 28: 297-304.
- (9) Dudhgaonkar S., Thyagarajan A., Sliva D. Suppression of the inflammatory response by triterpenes isolated from the mushroom *Ganoderma lucidum*. *Int Immunopharmacol.* 2009; 9 (11): 1272-80.
- (10) Park, J.E., Cuong, T.D., Hung, T.M., Lee, I, Na, M., Kim, J.C., Ryoo, S., Lee, J.H., Choi, J.S., Woo, M.H., Min, B.S. Alkaloids from *Chelidonium majus* and their inhibitory effects on LPS-induced NO production in RAW264.7 cells. *Bioorg Med Chem Lett.* 2011; 21 (23): 6960-3.
- (11) Shuai, H., Xian-li, Z., Cui-juan, W., You-song, W., Feng, X., Lian-hai, S., Zhi-yun, G., Jie, W. Pyrrolizidine alkaloids from *Liparis nervosa* with inhibitory activities against LPS-induced NO production in RAW264.7 macrophages. *Phytochem.* 2013; 93: 154-161.
- (12) Chang, G., Xiao-Xiao, H., Ming, B., Jie, W., Jian-You, L., Qing-Bo, L., Ling-Zhi Li, Shao-Jiang, S. Anti-inflammatory sesquiterpene pyridine alkaloids from *Tripterygium wilfordii*. *Fitoterapia* 2015; 105: 49-54.

## دراسة إمكانات مضادات الأكسدة ومضادات الالتهاب في الأجزاء الهوائية فيكوس نيتيدا (L.) موراسي وتركيبها الكيميائي النباتي

خالد راشد<sup>1</sup>، شينغ شولي<sup>2</sup>، مينهوسينغ بان<sup>3</sup>، كريستيان فيتوسا<sup>5</sup>

<sup>1</sup> قسم العقاقير، مركز البحوث الوطني، 33 شارع البحوث، الدقي، الجيزة، مصر، ص.ب. 12622

<sup>2</sup> قسم علوم الغذاء البحري، جامعة كاوسينغ البحرية الوطنية، كاوسينغ، تايوان

<sup>3</sup> مختبر هويباي كاي لتحسين المادة الوراثية للغابات الاقتصادية والاستغلال الشامل للمصادر، كلية العلوم الحياتية،

جامعة هونغانغ الطبيعية، هويباي، الصين

<sup>4</sup> معهد تكنولوجيا علوم الغذاء، جامعة تايوان الوطنية، تايبيه 10617، تايوان

<sup>5</sup> جامعة بياوي الاتحادية، مختبر النواتج الطبيعية، قسم الكيمياء، إنينجا، 64049-550، تيريسينا-باي، البرازيل.

### ملخص

الهدف من هذه الدراسة الحالية هو تقييم الأنشطة المضادة للأكسدة والمضادة للالتهابات من مستخلص الميثانول 80% من الأجزاء الهوائية من فيكوس نيتيدا ودراسة المكونات الكيميائية الرئيسية الموجودة في المستخلص النباتي.

تم اختبار المستخلص لمضادات الأكسدة باستخدام ديف ولإمكانات المضادة للالتهابات باستخدام مقايسة النترتريت وأيضا تم فحص مكونات المستخلص النباتي من خلال اختبارات كيميائية مختلفة. وأظهرت النتائج أن المستخلص له تأثير كبير كمضاد للأكسدة كمضاد للالتهابات، وأظهر المستخلص الميثانول من فيكوس نيتيدا مع تركيزات مختلفة تأثير ملحوظ كمضاد الأكسدة. وبشكل ملحوظ تثبيط إنتاج النترتريت وأثبت التحليل الكيميائي لمستخلص الميثانول وجود الكروبيدرات والعفصيات والفلافونويد والقلويدات والتربينات.

وأظهرت هذه النتائج أن المستخلص الميثانولي من الأجزاء الهوائية من فيكوس نيتيدا يمكن أن تكون بمثابة مرشح محتمل كمضاد للالتهابات.

**الكلمات الدالة:** فيكوس نيتيدا، الأجزاء الهوائية، مضادات الأكسدة، مضادات الالتهاب، المكونات الكيميائية.