International Food Research Journal 24(5): 2050-2058 (October 2017)
Journal homepage: http://www.ifrj.upm.edu.my

Total phenolic and flavonoid contents and free radical scavenging components of *Ficus nota* Merr. (Moraceae) ethanolic leaf extract

[1,2,3*]Santiago, L.A., [2]Saguinsin, S.G.C., [2]Reyes, A.M.L., [2]Guerrero, R.P., [2]Nuguid, A.M.N. and [3]Santos, A.C.N.

[1]*Research Center for the Natural and Applied Sciences, University of Santo Tomas, España Blvd., Manila, Philippines, P.O. 1015*
[2]*Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, España Blvd., Manila, Philippines, P.O. 1015*
[3]*The Graduate School, University of Santo Tomas, España Blvd., Manila, Philippines, P.O. 1015*

Article history

Received: 17 July 2016
Received in revised form: 31 August 2016
Accepted: 1 September 2016

Keywords

Ficus nota
Prooxidant
Antioxidant
Free radicals

Abstract

The leaves of *Ficus nota* or "tibig" are traditionally consumed as vegetable or used as herbal remedy. The primary aim of this research was to further evaluate the antioxidant and prooxidant nature of the plant by subjecting the ethanolic leaf extract containing alkaloids, flavonoids, phenols, terpenoids, saponins, and tannins to various general and specific antioxidant assays. Total phenolic and flavonoid content were found to be 348.3 ± 3.2 mg GAE/g and 2.64 ± 0.06 mg QE/g. The extract exhibited a concentration dependent inhibitory effect on DPPH, ABTS, and nitric oxide radical with an EC_{50} of 970.6, 269.8, and >5000 ppm respectively. Stimulation of superoxide, hydrogen peroxide, and hydroxyl radicals with median concentrations above 785.2 ppm was observed. The extract also showed a high dose dependent reducing capacity (EC_{50}= 548.3 ppm) and metal chelating potential (EC_{50}=27.53 ppm) towards iron. Results revealed that the plant extract possess both antioxidant and prooxidant properties depending on the concentration by which they are used. The dual nature exhibited by *F. nota* is deemed beneficial for the treatment and prevention of cardiovascular disease, diabetes, cancer, and aging. It can also aid in the maintenance of cellular homeostasis, host defense mechanisms, and various signaling processes in the body.

© All Rights Reserved

Introduction

Ficus nota (Blanco) Merr. or "tibig", from the Moraceae family, is a plant endemic to the Philippines. It is widely distributed from Luzon to Visayas where it originated from primary forests at low to medium elevations. The tree can grow up to 10 meters in height with prominent protuberances on the trunk. Leaves are simple, alternate, obovate, pubescent, and are approximately 24-28 x 12-14 cm in size (Tolones, 2003). The fruits are traditionally eaten with sugar and the leaves are consumed as vegetable. Different parts of the plant are ethnomedicaly used for the treatment of diabetes, hypertension, fever, urinary tract infection, and muscle pain (Polinag, 2003). The bioactivity of *F. nota* is one of the least explored amongst *Ficus* species. A study on the chemical composition of unripe fruits has shown the presence of 4-(2-hydroxyethyl)-2-methoxyphenol, (2R,3R)-2,3-butanediol, (2S,3S)-2,3-butanediol, and ß-sitosterol (Ragasa *et al*., 2014). Structural moieties of methoxyphenols and sitosterols are known to exhibit antioxidant, prooxidant, anti-inflammatory, antibacterial, hypocholesterolemic, anti-diabetic, cytotoxic, and immunomodulatory effects (Desai *et al*., 2009; Kadoma *et al*., 2010; Subramaniam *et al*., 2014; Murakami *et al*., 2015)

Previous study suggests that the *F. nota* methanolic leaf extract exhibits acetylcholinesterase inhibitory activity at low concentrations. It is also said to be inhibitory for both hydroxyl and nitric oxide radicals and stimulatory towards hydrogen peroxide (Ares *et al*., 2015). This antioxidant and prooxidant nature of *F. nota* is supported by several studies on related species. *Ficus fiskei* and *Ficus ulmifolia* were found to inhibit nitric oxide but shows stimulatory effects on superoxide and hydroxyl radicals whereas, *Ficus odorata* predominantly acts as a prooxidant in all tested conditions (Santiago and Mayor, 2014; Santiago and Balidoy, 2015; Santiago and De Rojas, 2015). The dual nature observed on some *Ficus* species may be influenced by the individual and synergistic response of the inherent lipophilic and hydrophilic bioactive compounds present in the plant extract. *Ficus* are generally composed of flavonoid glycosides, alkaloids, phenolic acids, steroids,

*Corresponding author.
Email: *santiagolibrado@yahoo.com*

saponins, coumarins, tannins, and triterpenoids (Sirisha *et al*., 2010). NMR spectroscopy revealed that the dichloromethane extract from the leaves of *Ficus pseudopalma* and *F. ulmifolia* contains squalene, polyprenol, β-amyrin fatty acid ester, α-amyrin fatty acid ester, α-amyrin acetate, β-amyrin acetate, lutein, lupeol acetate, β-carotene, phytol, sitosterol, and stigmasterol (Ragasa *et al*., 2009). Most of these compounds such as gallic acid, caffeic acid, lupeol, ß-amyrin, and ß-sitosterol have been reported to exhibit both stimulatory and inhibitory activities towards free radicals in a concentration dependent manner (Cordero *et al*., 2012). The primary aim of this research was to further evaluate the antioxidant and prooxidant nature of *F. nota* by subjecting the crude ethanolic leaf extract to various general and specific antioxidant assays, and to determine the presence of phytochemicals that may have contributed to its purported bioactivity. This study provides a comprehensive background on the dual property of *F. nota* that could aid in future pharmaceutical or nutraceutical applications of the plant.

Materials and Methods

All chemicals and standards used are analytical grade reagents purchased from Sigma Chemical Co. (Singapore). Corona Electric SH-1000 UV-VIS microplate reader was employed for all spectrophotometric measurements. Sorvall Biofuge Primo R centrifuge and Thermo Scientific thermo-mixer were also used (Thermo Fisher Scientific Inc., USA). Assays were all performed in triplicate (n=3) and adjusted to fit a micro-scale setting. Room temperature was maintained at 24 ± 2°C for all analyses. *F. nota* leaf extract was prepared in 80% ethanol at various concentrations. Gallic acid, quercetin, L-ascorbic acid and sodium EDTA were used as reference standards.

Plant preparation and extraction

Ficus nota leaves were authenticated at the Philippine National Museum. Leaves were thoroughly washed with distilled water to remove dust, soil, and other extraneous matter. Samples were subjected to air-drying at 25-30°C and were ground into fine powder with the use of a Wiley Mill apparatus. The crude extract was produced by repeated percolation using 80% ethanol with 1:10 sample-solvent ratio. Pooled extracts were concentrated in vacuo and stored at -20°C until further analysis.

Phytochemical analysis

Qualitative test tube method

Standard qualitative and colorimetric tests were done to detect the presence of bioactive metabolites such as alkaloids, flavonoids, saponins, terpenoids, and phenols in the crude ethanolic leaf extract (Aguinaldo *et al*., 2004). Mayer’s and Dragendorff’s test were used for the detection of alkaloids, froth test for saponins, Shinoda and alkaline reagent test for flavonoids, Salkowski’s test for terpenoids, gelatin test for tannins, and ferric chloride test for phenols.

Fast blue BB assay

The total phenolic content (TPC) of the extract was determined using the Fast Blue BB method described by Medina (2011). In a 96-well microplate, 200 µL of standard or sample was mixed with 20 µL of 0.1% Fast Blue BB reagent and 20 µL 5% sodium hydroxide. The mixture was allowed to equilibrate at room temperature for 90 minutes and the absorbance was measured at 420 nm. Results were expressed as milligram gallic acid equivalents per gram of extract (mg GAE/g).

Aluminum chloride assay

Total Flavonoids (TFC) were quantified based on the method used by Ordoñez *et al.* (2006) and Corpuz *et al.* (2013). One hundred microliters of standard or sample was added to 100 µL of 2% aluminum chloride. The mixture was allowed to stand at room temperature for 60 minutes. The absorbance was measured at 410 nm and the result was expressed as milligram quercetin equivalents per gram of extract (mg QE/g).

General evaluation of antioxidant activity

DPPH free radical scavenging

The capacity of the plant extract to scavenge DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radicals was evaluated in reference to the procedure used by Shen *et al.* (2010) with minor modifications. One hundred microliters of 0.1 mM DPPH in ethanol was added to 2 µL of ascorbic acid or sample. Absorbance at 517 nm was read every 30 minutes for a total of 120 minutes.

ABTS radical scavenging

The ability of the extract to inhibit ABTS [2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radicals was determined following the method used by Dhar *et al.* (2012) and Jan *et al.* (2013). The cation solution was prepared by mixing equivalent

amount of solution A and B. Solution A was made by dissolving 8 mg of ABTS in 1 mL ultrapure water whereas, 13.2 mg of potassium persulfate was added to 10 mL ultrapure water for solution B. The stock solution was allowed to equilibrate in the dark at room temperature for 16 hours before use. The working solution was freshly prepared by diluting the stock with 80% ethanol until an absorbance of 0.7-0.8 at 734 nm was reached. For the assay, 100 µL of the ABTS working solution was added to 10 µL of ascorbic acid or sample. The absorbance was measured every 5 minutes for 20 minutes.

Reducing power

The ferric to ferrous reduction in the presence of the antioxidant was monitored by measuring the formation of Perl's Prussian blue at 765 nm (Oyaizu, 1986; Barros *et al*., 2007). For the assay, 100 µL of ascorbic acid or sample was added to 100 µL of 0.2 M phosphate buffer (pH 6.6) and 100 µL of 1% potassium ferricyanide. The resulting mixture was placed in a thermo-mixer for 30 minutes at 50°C. One hundred microliters of 10% trichloroacetic acid was then added and the solution was centrifuged at 3000 rpm for 10 minutes. In a 96-well microplate, 100 µL of the upper layer was mixed with 100 µL ultrapure water and 20 µL of 0.1% fresh ferric chloride solution. The mixture was allowed to equilibrate at room temperature and the absorbance was read after 10 minutes. Higher absorbance indicates higher reducing power.

Metal chelating activity

The chelating property of the plant extract towards ferrous ions was determined as described by Dinis *et al*. (1994) with minor modifications. One hundred microliters of sodium EDTA or extract was mixed with 5 µL of 2 mM ferrous chloride. The solution was allowed to equilibrate for 5 minutes at room temperature. The reaction was then initiated by the addition of 20 µL 5 mM ferrozine. Absorbance was measured at 562 nm every 2 minutes for 10 minutes.

Inhibitory and stimulatory effect on specific free radicals

To fully elucidate the extract's full antioxidant or prooxidant capacity, tests evaluating effectiveness against various reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydroxyl radical, hydrogen peroxide, superoxide anion, and nitric oxide have been undertaken (Prior *et al*., 2005; Santiago and Valerio, 2013).

Nitric oxide radical scavenging

The nitric oxide scavenging capacity of the extract was determined by Griess reaction. One hundred microliters of the crude extract was mixed with 400 µL of 10 mM sodium nitroprusside and 100 µL phosphate buffered saline, pH 7.4. The resulting mixture was incubated for 150 min at room temperature. After incubation, 100 µL aliquots of each mixture were transferred to new tubes and subsequently mixed with 200 µL of 0.33% sulphanilamide. The solution was allowed to equilibrate for 5 minutes prior to the addition of 200 µL 0.1% napthylethylenediamine. The tubes were further incubated for 30 min. Aliquots of 250 µL were then transferred to a 96-well microplate and the absorbance was read at 540 nm.

Hydroxyl radical scavenging

In a microfuge tube, 200 µL of the extract at different concentrations were mixed with 20 µL of 0.1 mM EDTA, 2µL of 0.1 mM ferric chloride, 20 µL of 2 mM hydrogen peroxide, 72 µL of 3 mM deoxyribose, 66 µL phosphate buffer at pH 7.4, and 20 µL of L-ascorbic acid. The resulting mixtures were allowed to stand for 30 minutes at 37°C. After which, 100 µL of 5% trichloroacetic acid and 100 µL of 1% thiobarbituric acid was added. The tubes were placed in a 100oC water bath for 30 minutes. One hundred microliter aliquots were then transferred to a 96-well microplate and the absorbance was measured at 532 nm.

Superoxide radical scavenging

The ability of the extract to scavenge superoxide radicals was assessed by combining 5 µL of the extract with 50 µL of 73 µM reduced ß-nicotinamide adenine dinucleotide, 50 µL of 150 µM nitrobluetetrazolium, and 50 µL of 60 µM phenazine methosulfate. The absorbance was measured at 560 nm after 5 minutes of incubation.

Hydrogen peroxide scavenging

In a 96-well microplate, 80 µL of the extract at various concentrations were mixed with 120 µL of 40 mM hydrogen peroxide. The absorbance was read at 230 nm after 10 minutes.

Statistical analysis

Data gathered were reported as mean ± standard error of the mean. Single-factor analysis of variance (ANOVA) was used to determine significant differences at $p \leq 0.05$ using IBM SPSS Statistics version 21. Post-hoc analysis (Scheffe and Tukey's HSD) was also performed. Absolute EC_{50} values, concentrations producing 50% inhibition

or stimulation, were determined by plotting the percent mean activity over the log of concentration in the nonlinear regression curve fit function (viz., log of inhibitor against four parameters variable slope response, and least squares fitting method with interpolation at 95% confidence interval) of the Graph Pad Prism 6 software. Linear regression analysis using Microsoft Office Excel 2007 was used to estimate the total phenolic and flavonoid content of the plant extract by constructing standard calibration curves.

Results and Discussion

Phytochemical analysis

The crude ethanolic leaf extract of *F. nota* was positive for alkaloids, flavonoids, phenols, terpenoids, saponins, and tannins based on the results obtained from standard qualitative and colorimetric tests. The total phenolic and flavonoid content of the extract was quantified based on the linear regression equation derived from the gallic acid and quercetin calibration curve ($y=0.0001x + 0.034$, $R^2=0.992$ and $y=0.014x-0.020$, $R^2=0.998$ respectively). The TPC was found to be 348.3 ± 3.2 mg GAE/g whereas, results from the aluminum chloride assay showed a TFC of 2.64 ± 0.06 mg QE/g of extract.

Flavonoids, terpenoids, and other phenolic compounds are known to play major contributory roles in the antioxidant and prooxidant capacities exhibited by plant extracts. The antioxidant effect conferred by these compounds are due to the phenolic hydroxyl groups attached to their respective ring structures that can act as reducing agents, hydrogen donors, singlet oxygen quenchers, superoxide radical scavengers, and as metal chelators. They are also said to reduce α–tocopherol radicals or tocopheroxyls, activate antioxidant enzymes, mitigate nitrosative stress, and inhibit oxidases (Sakihama *et al*., 2002; Prochazkova *et al*., 2011). After proton donation, these compounds are oxidized to resonance-stabilized radicals that can further act as prooxidants at high concentrations, high pH, and in the presence of metal ions (Bravo, 1998; Sisein, 2014). In lieu to this, some of the most abundant flavonoids and phenolic acids present in food such as quercetin, myricetin, caffeic acid, gallic acid, chlorogenic acid, coumaric acid, ferulic acid, and ellagic acid have been proven to exhibit dual in vitro behavior (Simic *et al.*, 2007; Fukumoto and Mazza, 2009; Cotoras *et al*., 2014). Results from previous studies suggest that the identified triterpenes from F. *pseudopalma* such as α–amyrin, oleanolic acid, and ursolic acid can effectively act as proton donors and inhibitors of lipid peroxidation whereas, lupeol was

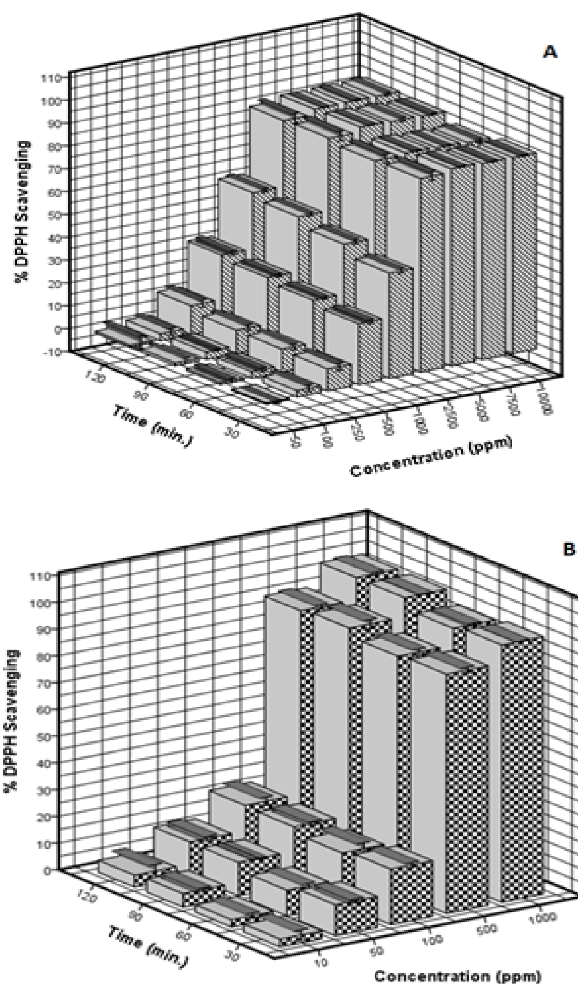

Figure 1. DPPH free radical scavenging capacity of *F. nota* (A), and ascorbic acid (B). Results are expressed as mean ± SEM of three determinations (n=3; p≤ 0.05).

found to exert stimulatory effects toward hydroxyl and superoxide radicals (Santiago and Mayor, 2014; Santiago *et al*., 2014). These compounds are also assumed to be responsible for the antioxidant and prooxidant properties exhibited by the crude ethanolic leaf extract of *F. nota.*

General

The antioxidant potential in terms of radical scavenging and chelating capacity was determined by monitoring the increase or decrease in the percentage activity as a function of time. DPPH radical is regarded as a model for lipophilic free radicals and is routinely used to assess the hydrogen donating ability of a substance. It generally relies on the reduction of the DPPH free radical in the presence of the antioxidant causing a colorimetric change from purple to yellow and a characteristic decrease in absorbance (Kalita *et al*., 2013; Formagio *et al*., 2014).

Results from the DPPH assay showed a concentration dependent scavenging effect that

Table 1. EC_{50} of *F. nota* and chemical standards on general antioxidant systems

Extract or Standard	EC50 values (ppm)			
	DPPH radical[a]	ABTS radical[b]	Metal chelating[c]	Reducing power[d]
F. nota leaf extract	970.6	269.8	27.53	548.3
L-ascorbic acid	191.9	74.30	-	57.94
Sodium EDTA	-	-	9.33	-

Absolute EC_{50} values are derived from non-linear regression analysis of the mean percent activity ± SEM of the extract and standard at n=3 (p≤0.05). EC_{50} is the concentration capable of exhibiting 50% inhibition at 60 min. (a), 5 min. (b), and 10 min. (c), whereas, (d) is the concentration producing an optical density of 0.5 at 765nm; (-) not determined.

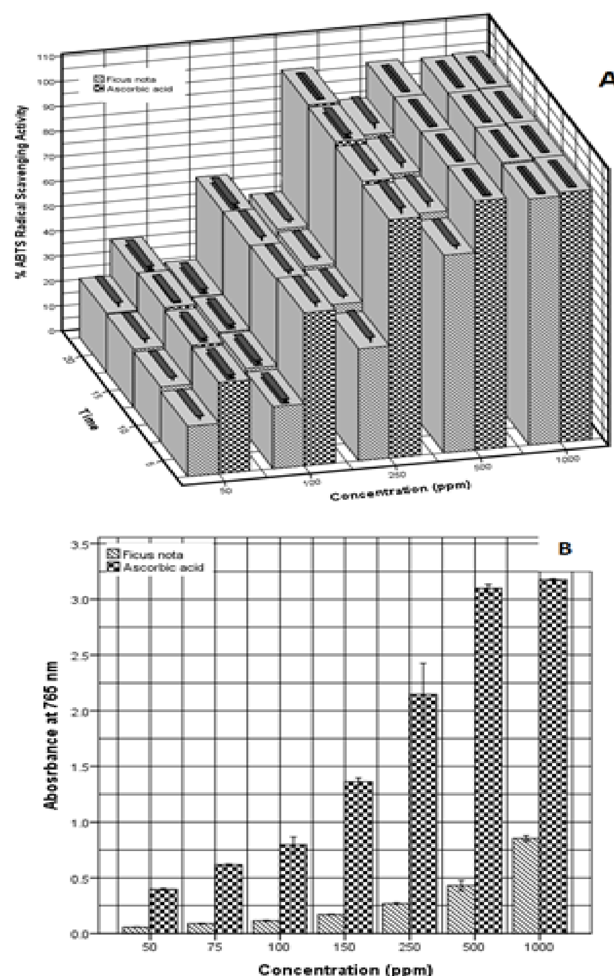

Figure 2. ABTS radical scavenging activity (A) and ferric reducing capacity (B) of *F. nota* and ascorbic acid. Results are expressed as mean ± SEM of three determinations (n=3; p≤ 0.05).

significantly increases over time at p≤0.05 as compared to the standard ascorbic acid which exhibited a constant scavenging capacity (Figure 1). Because of this, it is assumed that the plant extract probably contains slow reacting antioxidants. As indicated in Table 1, the IC_{50} (reported as EC_{50}) of the extract was not comparable to that of the standard. Nevertheless, post-hoc analysis revealed that the scavenging activity of the extract at high concentrations (2,500-10,000 ppm) matches the capacity of 500 ppm ascorbic acid which is commendable considering the crude nature of the sample.

Similarly, as illustrated in Figure 2A, the leaf extract and ascorbic acid also followed a concentration dependent inhibition of the ABTS radical with no significant increase within 5 to 20 minutes of exposure (p>0.05). Despite the obvious difference between the EC_{50} values of *F. nota* and vitamin C, Tukey's HSD and Scheffe's test at 95% confidence level proved that both are equally potent at high concentrations (750-2500 ppm). This assay is also based on the discoloration of the blue-green radical in the presence of the antioxidant. Although ABTS do not structurally resemble free radicals in the biological system, it is still considered efficient for the preliminary assessment of the hydrogen donating capability of a substance (Rehman *et al*., 2013).

For the reducing power assay, the antioxidant reduces the ferric/ferricyanide complex to ferrous form that can be quantified by measuring the formation of Perl's Prussian blue at ~700 nm. The reducing capacity of a substance is usually associated with the presence of reductones, such as polyphenols, which exert antioxidant effect by donating a hydrogen atom and by breaking the free radical chain (Gordon, 1990; Phang *et al.*, 2011). Figure 2B shows that the ethanolic leaf extract also follows a concentration dependent reducing potential. Post-hoc analysis suggests that the activity of the extract at 250-1000 ppm already compares to that of ascorbic acid (50-100 ppm).

Direct reaction is not the only mechanism by which antioxidants exert their effect. Preventive or secondary antioxidants can also decrease the rate of oxidation by chelating prooxidant metals. Iron, cobalt, copper, chromium, and other redox-active transition metals can promote oxidation by acting as catalysts in various free radical reactions or by transferring electrons during shifts in oxidation states. Substances with chelating properties reduce the prooxidant effects of these metals by decreasing their respective redox potentials, stabilizing their oxidized form, and by sterically hindering the formation of metal-hydrogen peroxide complexes (Reische *et al*., 2008; Koncic *et al*., 2011). The metal chelating assay measures the ability of the antioxidants to compete with ferrozine in chelating ferrous ions by

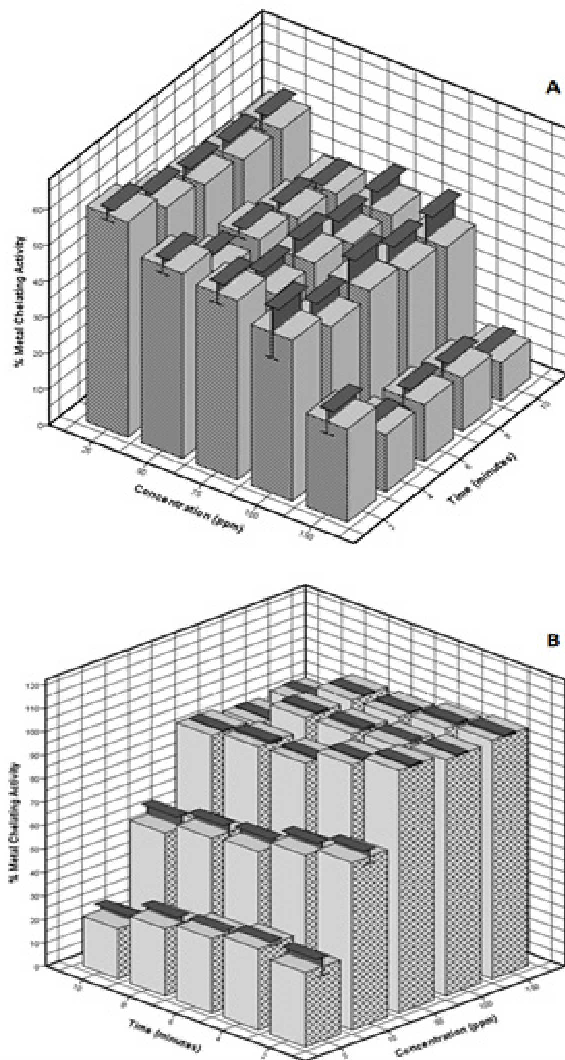

Figure 3. Metal chelating activity of F. nota (A), and sodium EDTA (B). Results are expressed as mean ± SEM of three determinations
(n=3; p≤ 0.05)

monitoring the rate of color reduction at 562 nm (Jayasri *et al*., 2009). At 27.53 ppm, the extract was already able to exhibit 50% metal chelation that is only three-folds higher than the EC_{50} established by the standard (Table 1). However as shown in Figure 3, the chelating potential of the extract was observed to be inversely related with concentration and time. This behavior may be due to the presence of both free and bound iron in the plant extract. The concurrent increase in the inherent iron content may have added to the amount of unbound ferrozine that was measured spectrophotometrically.

Data gathered from these assays suggest that the extract exhibits a concentration dependent and time consistent hydrogen donating and metal chelating properties. As summarized in Table 1, the *F. nota* ethanolic leaf extract can efficiently exert antioxidant effects at concentrations below 1000 ppm.

Inhibitory and stimulatory effect on specific free radicals

Free radicals or reactive oxygen species (ROS) normally participates in the maturation of cellular structures and serve as weapons for several host defense mechanisms (Pham-Huy *et al*., 2008). However in excess, these ROS creates oxidative stress that disrupts the balance among inherent prooxidant-antioxidant reactions in living organisms. The uncontrollable rise in intracellular ROS concentration causes damage in lipids, proteins, and DNA that subsequently leads to the progression of various chronic diseases. Natural and synthetic substances that exhibits dual regulatory properties depending on the concentration by which they are used can help maintain or restore redox homeostasis in the body. Recently, the "cross-over" effect possessed by these substances has also been viewed beneficial for the prevention of carcinogenesis through their cytotoxic and apoptotic response against cancer cells (Buettner and Jurkiewicz, 1996; Cordero *et al*., 2012). The activity of the *F. nota* ethanolic leaf extract against free radicals (NO•, $•O_2^-$, H_2O_2, and •OH), was evaluated in this study.

Nitric oxide is an important chemical mediator produced in biological tissues by the metabolism of arginine to citruline in the presence of nitric oxide synthase (NOS). It is necessary for a large variety of physiological processes such as neurotransmission, blood pressure regulation, host defense mechanisms, smooth muscle relaxation, and immunoregulation (Ghafourifar and Cadenas, 2005; Sisein, 2014). Conversely, excess production and subsequent reaction of nitric oxide with superoxide anion can create more oxidatively damaging molecules like peroxynitrites that can further lead to DNA fragmentation and lipid oxidation (Valko *et al*., 2007). As shown in Figure 4, the extract was able to exhibit a dose dependent inhibitory effect on nitric oxide radical with 50% scavenging capacity at concentrations above 5000 ppm. This activity may be due to the observed hydrogen donating ability of the extract as influenced by its phenolic and flavonoid contents (Nijveldt *et al*., 2001).

On the other hand, it was observed that the ethanolic leaf extract also acts as a prooxidant towards superoxide, hydrogen peroxide, and hydroxyl radicals with 50% stimulatory effect at >5000 ppm, 4447 ppm, and 785.2 ppm respectively (Figure 4). These observed activities somehow support the results obtained from previous study (Ares *et al*., 2015). Superoxide anion is a reduced form of molecular oxygen that is produced from mitochondrial transport systems. It is involved in the formation of other free

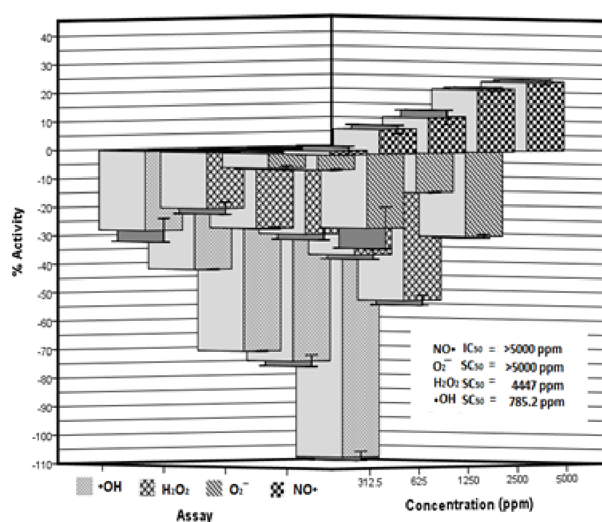

Figure 4. Inhibitory and stimulatory effect of *F. nota* leaf extract on specific free radicals. Results are expressed as mean ± SEM of three determinations
(n=3; p≤0.05).

radicals such as singlet oxygen, hydroxyl radical, and hydrogen peroxide. The latter is considered to be the least reactive among ROS and is stable under physiological conditions in the absence of metal ions. Both radicals play an important role in the immune system by killing invading microorganisms during phagocytosis and by acting as secondary messengers. These radicals are also said to possess cellular modulating functions. Hydrogen peroxide was reported to mimic the action of insulin growth factor and act as a mitogen *in vitro* (Devasagayam et al., 2004). Hydroxyl radical as produced by the Haber-Weiss and Fenton reaction is considered to be the most biologically active and damaging among ROS. Nevertheless, their reactive nature helps promote the action of macrophages and assists in maintaining cellular homeostasis (Lipinski, 2011; Santiago and Mayor, 2014).

The prominent and distinct prooxidant and antioxidant property of the plant extract can be due to the balance between its free radical scavenging and reducing capacity on iron. During the Fenton reaction, the ferrous ion reacts with hydrogen peroxide to form hydroxyl radicals. Initially, ferrous is oxidized to ferric but in the presence of reductones, the ions can be easily transformed back to its reduced state hence, contributing to the enhanced generation of hydroxyl radicals as evident in Figure 4. The predominant reducing capacity (EC_{50}=548.3 ppm) of the extract over its free radical scavenging property (EC_{50}=970.6 ppm), alongside its probable high inherent iron content, may have influenced the prooxidant effect of the extract against ROS (Tian and Hu, 2005; Ling *et al.*, 2010). As indicated in Figure 4, the *F. nota* ethanolic leaf extract possess an inhibitory effect against nitric oxide and stimulatory effect on hydrogen peroxide, superoxide, and hydroxyl radicals when used at high concentrations. These findings are crucial and should be taken into account for future product formulations and other medicinal or food applications of the plant.

Conclusion

The dual property of the *F. nota* ethanolic leaf extract was assessed to establish its effectiveness as an antioxidant and/or prooxidant. Phytochemical analysis indicated the presence of alkaloids, flavonoids, phenols, terpenoids, saponins, and tannins that may have significantly contributed to its purported bioactivity. Results from general and specific antioxidant assays revealed that the plant extract acts as an antioxidant at low concentrations and exhibits prooxidant effects at high concentrations. In this regard, *F. nota* can be considered as an agent with dual potential in biological and pharmaceutical applications that is deemed beneficial for the treatment and prevention of cardiovascular disease, diabetes, cancer, and aging. Additionally, the two-edge nature exhibited by the plant extract can also aid in the maintenance of cellular homeostasis, host defense mechanisms, and various signaling processes in the body.

Acknowledgement

The researchers thank Ms. Regina B. Callanta for support and assistance.

References

Aguinaldo, A.M., Espeso, E., Guevara, B.Q. and Nonato, M.G. 2004. Phytochemistry. In Guevara, B.Q. (Ed). A Guidebook to Plant Screening: Phytochemical and Biological, p. 24-69. Philippines: University of Santo Tomas Publishing House

Ares, K.G.D., Capuno, C.V.F., Cortes, H.K.M., Cristi, A.T.L., Cutero, H.C.L., Galigao, G.P., Santiago, L.A. and Sy, S.C. 2015. In vitro assessment of the antioxidant and acetylcholinesterase inhibitory activity of *Ficus nota* (Blanco) Merr. International Journal of Pharmacy 5(4): 1020-1025.

Baig, H., Ahmed, D., Zara, S., Aujla, M.I. and Asghar, M.N. 2011. In vitro evaluation of antioxidant properties of different solvent extracts of *Rumex acetosella* leaves. Oriental Journal of Chemistry 27(4): 1509-1516.

Barros, L., Ferreira, M.J., Queiros, B., Ferreira, I.C.F.R. and Baptista, P. 2007. Total phenols, ascorbic acid, ß-carotene and lycopene in Portuguese wild edible

mushrooms and their antioxidant activities. Food Chemistry 103: 413-419.

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56: 317-333.

Buettner, G. and Jurkiewicz, B.A. 1996. Catalytic metals, ascorbate, and free radicals: combinations to avoid. Radiation Research 145: 532-541.

Cordero, C.M., Gonzalez, A.J.L., Montaño, J.M.C., Moron, E.B. and Lazaro, M.L. 2012. Pro-oxidant natural products as anticancer agents. Current Drug Targets 13(8): 1006-1028.

Corpuz, M.J.A.T., Osi, M.O. and Santiago, L.A. 2013. Free radical scavenging activity of *Sargassum siliquosum* J.G. Agardh. International Food Research Journal 20(1): 291-297.

Cotoras, M., Vivanco, H., Melo, R., Aguirre, M., Silva, E. and Mendoza, L. 2014. In vitro and *in vivo* evaluation of the antioxidant and prooxidant compounds obtained from grape *(Vitis vinifera)* pomace. Molecules 19: 21154-21167.

Desai, F., Ramanathan, M., Fink, C.S., Wilding, G.E., Guttman, B.W. and Awad, A.B. 2009. Comparison of the immunomodulatory effects of the plant sterol ß-sitosterol to simvastatin in peripheral blood cells from multiple sclerosis patients. International Immunopharmacology 9(1): 153-157.

Devasagayam, T.P.A., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.J.S. and Lele, R.D. 2004. Free radicals and antioxidants in human health: current status and future prospects. Journal of the Association of Physicians of India 52: 794-804.

Dhar, P., Tayade, A.B., Saurav, S.K., Chaurasia, O.P., Srivastava, R.B. and Singh, S.B. 2012. Antioxidant capacities and phytochemical composition of *Hippophae rhamnoides* L. leaves methanol and aqueous extracts from trans-Himalaya. Journal of Medicinal Plants Research 6(47): 5780-5788.

Dinis, T.C.P., Madeira, V.M.C. and Almeida, M.L.M. 1994. Action of phenolic derivatives (acetoaminophen, salycilate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics 315: 161-169.

Formagio, A.S.N., Volobuff, C.R.F., Santiago, M., Cardoso, C.A.L., Vieira, M.C and Pereira, Z.V. 2014. Evaluation of antioxidant activity, total flavonoids, tannins, and phenolic compounds in *Psychotria* Leaf Extracts. Antioxidants 3: 745-757.

Fukumoto, L. and Mazza, G. 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. Journal of Agricultural and Food Chemistry 48 (8): 3597-3604

Ghafourifar, P. and Cadenas, E. 2005. Mitochondrial nitric oxide synthase. Trends in Pharmacological Sciences 26: 190-195.

Gordon, M.F. 1990. The mechanism of antioxidant action in vitro. In Hudson, B.J.F. (Ed). Food Antioxidants, p. 1-18. London: Elsevier Applied Science

Jan, S., Khan, M.R., Rashid, U. and Bokhari, J. 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health Research Perspectives 4(5): 246-254.

Jayasri, M.A., Mathew, L. and Radha, A. 2009. A report on the antioxidant activity of leaves and rhizomes of *Costuspictus Don.* International Journal of Integrative Biology 5(1): 20-26.

Kadoma, Y., Murakami, Y., Ogiwara, T., Machino, M., Yokoe, I. and Fujisawa, S. 2010. Radical scavenging activity and cytotoxicity of ρ-methoxyphenol and ρ-cresol dimmers. Molecules 15: 1103-1112

Kalita, P., Tapan, B., Pal, T. and Kalita, R. 2013. Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. Journal of Drug Delivery and Therapeutics 3(4): 33-37.

Koncic, M.Z., Barbaric, M., Perkovic, I. and Zorc, B. 2011. Antiradical, chelating and antioxidant activities of hydroxynamic acids and hydroxyureas. Molecules 16: 6232-6242.

Ling, L.T., Palanisamy, U.D. and Cheng, H.M. 2010. Prooxidant/antioxidant ratio (ProAntidex) as a better index of net free radical scavenging potential. Molecules 15: 7884-7892.

Lipinski, B. 2011. Hydroxyl radical and its scavengers in health and disease. Oxidative Medicine and Cellular Longetivity. Retrieved on December 23, 2015 from Hindawi website: *http://www.hindawi.com/journals/omcl/2011/809696/cta*

Medina, M. 2011. Determination of the total phenolics in juices and superfruits by a novel chemical method. Journal of Functional Foods 3: 79-87.

Murakami Y., Kawata, A., Katayama, T. and Fujisawa, S. 2015. Anti-inflammatory activity of the artificial antioxidants 2-tert-butyl-4-methoxyphenol (BHA), 2,6-di-tert-butyl-4-methoxyphenol (BHT) and 2,4,5-tri-tert-butylphenol (TBP) and their various combinations. *In Vivo* 29 (2): 197-206

Nijveldt, R.J., Nood, E.V., Hoorn, E.C.V., Boelens, P.G., Norren, K.V. and Leeuwen, P.A.M.V. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. American Journal of Clinical Nutrition 74: 418-425.

Ordoñez, A.A., Gomez, J.G., Vattuone, M.A. and Isla, M.I. 2006. Antioxidant activities of *Sechium edule* swart extracts. Food Chemistry 97: 452-458

Oyaizu, M. 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 44: 307-315.

Pham-Huy, L.A., He, H. and Pham-Huy, C. 2008. Free radicals, antioxidants in disease and health. International Journal of Biomedical Science 4(2): 89-96.

Phang, C.W. Malek, S.N.A., Ibrahim, H. and Wahab, N.A. 2011. Antioxidant properties of crude and fractionated extracts of *Alpinia mutica* rhizomes and their total phenolic content. African Journal of Pharmacy and Pharmacology 5(7): 842-852.

Polinag, M.A. 2003. Food from the wilderness. DENR Recommends, p. 17-18. Philippines: Ecosystems Research and Development Bureau, Department of Environment and Natural Resources.

Prior, R.L., Wu, X. and Schaich, K. 2005. Standardized methods for the development of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53(10): 4290-4302.

Prochazkova, D., Bousova, I. and Wilhelmova, N. 2011. Antioxidant and prooxidant properties of flavonoids. Fitoterapia 82: 513-523.

Ragasa, C.Y., Alimboyoguen, A.B. and Shen, C.C. 2014. Chemical constituents of *Ficus nota*. Journal for Medicinal Chemistry, Pharmaceutical Chemistry, and Computational Chemistry 6(4): 98-101.

Ragasa, C.Y., Tsai, P.W. and Shen, C.C. 2009. Terpenoids and sterols from the endemic and endangered Philippine trees, *Ficus pseudo* palma and *Ficus ulmifolia*. Philippine Journal of Science 138(2): 205-209.

Rehman, J., Khan, I.U. and Asghar, M.N. 2013. Antioxidant activity and GC-MS analysis of *Grewia optiva.* E3 Journal of Biotechnology and Pharmaceutical Research 4(1): 14-21.

Saeed, N., Khan, M.R. and Shabbir, M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. Biomed Central Complimentary and Alternative Medicine 12: 221.

Sakihama, Y., Cohen, M., Grace, S. and Yamasaki, H. 2002. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology 177: 67-80.

Santiago, L.A. and Balidoy, S.B. 2015. Prooxidant and Antioxidant Polar Phytoconstituents from Endemic Philippines *Ficus fiskei* Elm. (Moraceae). International Journal of Pharmacy Teaching and Practices 6(2): 2127-2135.

Santiago, L.A. and De Rojas, B.N.S. 2015. Antioxidant/pro-oxidant action of Philippine endemic and endangered *Ficus ulmifolia* Lam. (Moraceae) Ethanolic Leaf Extract. International Journal of Pharmacy and Integrated Life Sciences 3(8): 51-68.

Santiago, L.A., Dayrit, K.C., Correa, P.C.B. and Mayor, A.B. 2014. Comparison of antioxidant and free radical scavenging activity of triterpenes α-amyrin, oleanolic acid, and ursolic acid. Journal of Natural Products 7: 29-36.

Santiago, L.A. and Mayor, A.B. 2014. Lupeol: An antioxidant triterpene in *Ficus pseudopalma* Blanco (Moraceae). Asian Pacific Journal of Tropical Biomedicine 4(2): 109-118.

Santiago, L.A. and Mayor, A.B. 2014. Prooxidant effect of the crude ethanolic leaf extract of *Ficus odorata* Blanco Merr. in vitro: It's medical significance. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering 8(1): 53-60.

Santiago L.A. and Valerio, V.L. 2013. Assessment of antioxidant activities of crude ethanolic leaf extract of *Ficus pseudopalma* Blanco (Moraceae). International Journal of Pharmaceutical Frontier Research 3(1): 1-11.

Shen, Q., Zhang, B., Xu, R., Wang, Y., Ding, X. and Li, P. 2010. Antioxidant activity *in vitro* of selenium-contained protein from the selenium enriched Bifidobacterium animalis 01. Anaerobe 16: 380-386.

Simic, A., Manojlovic, D., Segan, D. and Todorovic, M. 2007. Electrochemical behavior and antioxidant and prooxidant activity of natural phenolics. Molecules 12: 2327-2340.

Sirisha, N., Sreenivasulu, M., Sangeeta, K. and Chetty, C.M. 2010. Antioxidant properties of *Ficus* species-a review. International Journal of Pharmtech Research 2(4): 2174-2182.

Sisein, E.A. 2014. Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences 2(2): 110-118.

Subramaniam, S., Keerthiraja, M. and Sivasubramanian, A. 2014. Synergistic antibacterial action of ß-sitosterol-D-glucopyranoside isolated from *Desmostachya bipinnata* leaves with antiobiotics against common human pathogens. Revista Brasileira de Farmacognosia 24(1): 44-50.

Tian, B. and Hu, Y. 2005. Concentration-dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. Food Chemistry 91: 413-418.

Tolones, A. 2003. Isolation and structure elucidation of antitubercular constituents from the leaf extract of *Ficus nota* (Blanco) Merr. Manila, Philippines: University of Santo Tomas, MSc thesis.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology 39: 44-84.