



Prooxidant and Antioxidant Polar Phytoconstituents from Endemic Philippines *Ficus fiskei* Elm. (Moraceae)

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Research Article

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Abstract

It is a pioneering study providing referential pharmacobotanical, phytochemical information and potential medicinal use of *Ficus fiskei* Elmer, one of the 150 endemic *Ficus* species to the Philippines which to this day remains largely unutilized. This study aimed to assess its phytoconstituents profile, total polyphenols, total flavonoids, and prooxidant/antioxidant capacity using: phytochemical screening, fast blue BB method, $AlCl_3$ method, DPPH assay, ferric reducing antioxidant power (FRAP) assay, and specific antioxidant assays against superoxide (O_2^-), hydroxyl radical ($\cdot OH$), and nitric oxide ($NO\cdot$). The ethanolic leaf extract was positive for alkaloids, saponins, flavonoids, tannins and phenolics. The total phenolic content (TPC) and total flavonoid (TFC) were estimated at 0.019 mg gallic acid equivalent / mL sample and 0.635 mg quercetin equivalent / mL sample, respectively. TLC on silica gel G exposed seven spots, whereas one corresponded to that of standard quercetin with an R_f value of 0.63 in solvent system comprising toluene: ethyl acetate::methanol (6:3:2). Based on FRAP assay, the extract has high reducing capacity on iron ranging from 44-86%. Meanwhile, the crude extract is inhibitory on DPPH radical ($IC_{50} = 2.2$ mg/mL) and nitric oxide radical ($IC_{50} > 10$ mg/mL). Conversely, the extract is stimulatory on producing superoxide ($SC_{50}=0.786$ mg/mL), and hydroxyl ($SC_{50}=0.056$ mg/mL) radicals.

The findings suggest that *F. fiskei* acts as an antioxidant and prooxidant by modulating the production of reactive oxygen and nitrogen species which are important in neutralizing oxidative stress and maintaining human health..

Keywords: Prooxidant, Antioxidant, *Ficus fiskei* Elm., Reactive oxygen species, Reactive nitrogen species, Flavonoids

Introduction

There are over 800 genus *Ficus* in the tropics and subtropics that comprise the fig or mulberry family (Moraceae). The figs are deciduous and fast growing single-trunk trees among which of them, 150 species are endemic to the Philippines [1]. Studies on several *Ficus* species demonstrated radioprotective, hypocholesterolemic, gastroprotective, anti ulcerogenic, anti-inflammatory and hepatoprotective, antioxidant, antibacterial activities, among others. These activities were largely attributed to the presence of phenolic compounds (gallic acid and ellagic acid), flavonoids, tannins, steroids, glycosides and coumarins [2] and pentacyclic triterpenes such as oleanolic acid, ursolic acid and α -amyrin acetate [3].

Depending on the circumstance, a compound may exhibit prooxidant or antioxidant activity. Examples include polyphenols, thiols, α -tocopherol and ascorbate. Since all plants contain polyphenols and flavonoids, they are the most known sources of natural antioxidants. Hence, interest on researches about naturally occurring antioxidants which protect the cells from reactive oxygen species (ROS) and reactive nitrogen species (RNS) that may trigger risk of degenerative diseases and other health conditions are greatly increased. Moreover, plants can also exhibit prooxidant activity which is linked to the apoptotic function important in the prevention of tumor progression and other biological reactions in the body [4]. Both superoxide and hydroxyl radicals are biologically produced by phagocytic cells as natural defense against invading foreign bodies [5].

A previous study reported that 15 *Ficus* species were cultivated at Mount Palay-palay, Mataas na Gulod



National Park including *Ficus fiskei* Elmer [6]. It is a native small tree of four meters tall distributed throughout in thickets and forests and moist localities at low and medium altitudes. Endemic to the Philippines, it is known locally as *Upling gubat* or *Isis* in Tagalog. It has a tough brown bark with fine spinulose hairs arising from the minute cones. The leaves are dry, quite brittle and equilateral. The leaves with the length of 22 cm and the width of 18 cm are distichously unequally arranged. The leaves have unequally subordinate bases, acute or acuminate apices, sparsely scabrous upper surfaces and in volute, undulate margins. The yellowish green veins and reticulations are prominent beneath the leaves. The uniformly short, stout, hispid, and acuminate sub glabrous petioles are usually 8mm long. The plant has a green young syconium and yellow-red ripen syconium. [6,7]



Figure 1. Photos of *Ficus fiskei* Elm. found at Sto. Domingo Albay, Bicol Province, Philippines. Original photos taken in June 2014.

The dried matured leaves of *F. fiskei* are hard and rough thus used for filing by the Ifugao in Northern Luzon as native sandpaper [8]. Other than this, a little is written about the plant neither its ethnomedicinal use, pharmaco-botanical nor phytochemical information. As a consequence, *F. fiskei* Elmer to this day remains understudied, underutilized, undervalued, and neglected. There is a great deal of interest in expanding its commercial utilization in food and pharmaceutical industry because of its potentials. Therefore, this preliminary study was undertaken to search its phytochemicals, assess its antioxidant, prooxidant and antibacterial activities, and open new possibilities.

Material and Method

Chemicals

Analytical grade ethyl alcohol used for the extraction of *F. fiskei* leaves was ordered from

Belman Laboratories (Philippines). The reagents, solvents, and standards were obtained from Sigma-Aldrich Co. (Singapore).

Plant collection and preparation

Ficus fiskei leaves were procured locally at Sto. Domingo Albay, Bicol Province, Philippines on the month of June 2014 and authenticated in the Botany Division, National Museum. The fresh leaves of *F. fiskei* were allowed to be air-dried for 7-10 days at room temperature. Moreover, the leaves were protected from the sunlight to avoid decomposition of labile compounds. The dried leaves were then pulverized into fine powder using Wiley Mill grinder. The powdered sample (549.3 g) was saturated seven times with 95% ethyl alcohol (solvent-sample ratio of 1:10) in a percolator until the solution become colorless. The filtrate was then concentrated using a rotary evaporator at 40°C until syrupy consistency was obtained. The extract was further evaporated to dryness for a few days. The crude extract was weighed, obtaining 5.02% yield and kept in amber-colored container.

Qualitative Colorimetric Phytochemical screening

Phytochemical screening of the crude ethanolic leaf extract was done accordingly as what were described [9]. For alkaloids – sample preparation; 0.5 g of extract was stirred with 5mL 1% aqueous hydrochloric acid on a water bath, and filtered. Dragendorff's test; 1mL of freshly prepared Dragendorff's reagent was added to the 1mL plant extract and observed for the formation of orange precipitate. Wagner's test; 1mL of Wagner's reagent was added to another 1mL of the plant extract and observed for the formation of reddish-brown precipitate. For glycosides- Modified Borntrager's test; 1% plant extract was evaporated to dryness through steam bath. A 10 mL 0.5M potassium hydroxide and 1mL 5% hydrogen peroxide were added. The mixture was heated over a steam bath before it was filtered. The residue was discarded. The solution was acidified using glacial acetic acid. Acid filtrate extraction was done twice with 5mL portions of benzene. Ammonia was added to the collected extract. The color produced by the solution after shaking was observed. For tannins – gelatin test; 1% gelatin solution in 10% NaCl was added to the 1% crude ethanolic extract. Formation of jelly precipitate indicates the presence of tannin. For saponins – foam test; 1mL of 1% plant extract was diluted with an equal amount of distilled water. The solution was shaken vigorously for 15



minutes. Stable foam of about 1cm confirmed the presence of saponin. For flavonoids - Shinoda's test; three pieces of Magnesium chips were added to the ethanolic extract of the plant and a few drops of concentrated hydrochloric acid were also added to observe a colored solution. Sodium Hydroxide test; 2mL of the plant extract was dissolved in 10% aqueous sodium hydroxide solution. Addition of dilute HCl was carried out after filtering the solution. A change in color in the solution was observed confirming the presence of flavonoid. For polyphenols – ferric chloride test; 1% crude ethanolic plant extract was prepared before adding hot distilled water. The upper layer was collected and three drops of 10% sodium chloride were added. The mixture was filtered before adding several drops of freshly prepared 10% ferric chloride solution. Colored solution was observed for the presence of phenol.

Thin layer chromatography

The components of the plant were separated and identified using the solvent system: toluene: ethyl acetate: methanol (6:3:2). The pre-coated TLC plate was first activated in an oven at 50°C for 30 minutes. The extract was spotted on the TLC plate together with the reference standard antioxidants: gallic acid, ellagic acid, quercetin, rutin, and oleanolic acid. The plates developed and the spots were visualized to compute the R_f value.

Estimation of Total Phenolic Content using Fast Blue BB microscale

Fast Blue BB [4-benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi (zinc chloride) salt] (FBBB) was used to measure the total phenolic content of the plant extract as described in the procedure [10]. In 0.1% plant extract, 0.1% FBBB reagent was added and was mixed for 30 second before the addition of 5% NaOH. The mixture was incubated for 90 minutes at room temperature before reading the absorbance at 420nm using microplate reader. The calibration standard curve was made from different concentrations of gallic acid to determine the phenolic concentration of the plant extract.

Estimation of Total Flavonoid Content using aluminum chloride test microscale

The Aluminum Chloride Method is a colorimetric assay that was used to measure the

total flavonoid content of the extract as cited in the procedure [10]. A mixture of 0.1mL with 0.1% plant extract, 0.3mL distilled water, and 0.03mL of 5% sodium nitrite was made. The mixture was incubated for 5 minutes at 25°C. An addition of 0.03mL of 10% aluminum chloride to the mixture was done before it was treated with 0.2mL of 1mM NaOH and diluted with 1mL distilled water. The absorbance was recorded at 510nm with distilled water used as the blank in a 96-well microplate. The calibration standard curve was made from the different concentrations of quercetin.

Microscale antioxidant tests

Each antioxidant assay was done in triplicates. The absorbances were measured using the Corono Electric, SH-1000 Microplate reader. The procedures for the assays were performed according to the discussed procedures [9,10,11,12]

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay

The hydrogen-donating activity of the plant to the DPPH radical was measured using this general assay. Different concentrations of the extract were prepared. Mixtures of 10µL of the different concentrations of the extract and 190µL of 6.58x10⁻³ DPPH solution dissolved in methanol were placed in 96-well plates. These mixtures were incubated at room temperature for 15 minutes in a dark room. Absorbance was recorded at 517nm. Calculation of the percentage scavenging effect was done using this formula:

$$\text{DPPH Scavenged (\%)} = \frac{[(\text{Abs}_{\text{DPPH}_{\text{RB}}}) - (\text{Abs}_{\text{S+R}})]}{\text{Abs}_{\text{RB}}} \times 100$$

Where: RB = Reagent without sample

S + R = Reagent with sample

FRAP (Ferric Reducing Antioxidant Power) assay

FRAP assay was used to measure the reducing power of the *Ficus fiskei* extract. Different concentrations of the extracts were prepared in 70µL Ependorff tubes. The extracts were mixed with 176.5 µL of 0.2M sodium phosphate buffer (pH 7.4) and 176.5 µL (1%) of potassium ferricyanide. These mixtures were incubated at 50°C for 20 minutes then; the mixtures were acidified with 176.5 µL of 10% trichloroacetic acid. The mixtures were centrifuged at 650 x g for 10 minutes. The 273 µL of the supernatant was added to 273 µL of deionized water. Lastly, 55 µL of 0.1 % FeCl₃ was added to the solutions. 200 µL of each solution was placed in a 96-well microplate. The absorbance was



recorded at 540nm to determine the percentage reduction of the extract using this formula:

$$\text{Reduction (\%)} = \frac{[(\text{Abs}_{\text{S+R}}) - (\text{Abs}_{\text{DPPH RB}})]}{(\text{Abs}_{\text{S+R}})} \times 100$$

Where: RB = Reagent without sample
S + R = Reagent with sample

Superoxide radical scavenging assay

This assay was used to determine the activity of the crude extract to scavenge the superoxide radicals. Different concentrations of the crude ethanolic plant extract were prepared. Five microliter of each extract were loaded in the microplate before the addition of 50 μl 73 $\mu\text{mol/L}$ NaDH, 50 μl 156 $\mu\text{mol/L}$ of nitrobluetetrazolium, and 50 μl 60 $\mu\text{mol/L}$ phenazine methosulfate were done. The mixtures were incubated for 5 minutes at 25°C. Absorbance was recorded at 560nm to compute for the % scavenging effect:

$$\text{Scavenging effect (\%)} = \frac{[(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

Where: A_{control} = absorbance of the control
 A_{sample} = absorbance of the test compound

Hydroxyl radical scavenging assay

In Hydroxyl radical scavenging assay, different concentrations of the plant extract were prepared before 2.4 mL of phosphate buffer (0.1M, pH 7.2) and 0.6 mL of H_2O_2 solution (40mM) were added to each extract. The mixtures were shaken vigorously and incubated for 10 minutes at room temperature. Reaction mixture absorbance was recorded at 230nm and the scavenging activity was computed using this formula:

$$\text{Scavenging effect (\%)} = \frac{[(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

Where: A_{control} = absorbance of the control
 A_{sample} = absorbance of the test compound

Nitric oxide radical scavenging assay

The assay was based on the method where sodium nitroprusside generates nitric oxide interacts with oxygen to produce nitrite ion. Mixtures of the different concentrations of the plant extract with sodium nitroprusside (10mM) in phosphate buffered saline were prepared. These mixtures were incubated for 150 minutes at room temperature. Griess reagent was added to each mixture. Absorbance was recorded at 546nm in

the microplate reader and the scavenging activity was calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{[(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

Where: A_{control} = absorbance of the control
 A_{sample} = absorbance of the test compound

Statistical Analysis

The results obtained from this study was expressed as mean \pm SEM. One-Way Analysis of Variance (ANOVA) with Tukey's HSD post-hoc analysis were used to determine if there is a significant difference in the mean percentage inhibition or stimulation on assays (DPPH, superoxide, nitric oxide and hydroxyl) and mean percentage reducing power using FRAP assay. P-values less than 0.05 indicate significant difference. Regression analysis was also done in the determination of total phenolic and flavonoid content in the sample.

Results and Discussion

This is a pioneering work that provides pharmacological use and phytochemical information on one of the endemic *Ficus* species to the Philippines, *Ficus fiskei* Elmer. Apart from its lone use as a native sandpaper, not much has been reported on the plant.

Physicochemical characteristics of the extract

The crude ethanolic leaf extract of *Ficus fiskei* Elm. contains alkaloids, tannins, saponins, flavonoids, and polyphenols as shown in Table 1. Flavonoids and polyphenols are well known antioxidants. In fact, *Ficus* species are widely reported to have antioxidant property [2]. Little do we know that some of them may be pro-oxidant like some of the green tea polyphenols [4].

Table 1. Results of phytochemical screening of the crude ethanolic leaf extract of *Ficus fiskei* Elmer

Plant Constituent	Qualitative test	Positive Result	Actual Result
Alkaloid	Dragendorff's test Wagner's test	Orange precipitate Reddish-brown precipitate	(++) presence of orange precipitate (+) presence of brown precipitate
Glycoside	Modified Borntrager's test	Pink color	(-) yellow-green solution
Tannin	Gelatin test	Jelly precipitate	(+) presence of jelly precipitate



Saponin	Foam test	Stable foam	(+) 1 cm foam
Flavonoid	Shinoda's test Sodium Hydroxide test	Red color Yellow color	(++) red solution (+) yellow solution
Polyphenol	Ferric chloride test	Blue-black precipitate or	(+) blue-black precipitate

Using the solvent system: toluene: ethyl acetate: methanol (6:3:2), thin layer chromatography was done against the known standards namely gallic acid, ellagic acid, quercetin, rutin, and oleanolic acid. Seven spots were observed in the TLC chromatogram of *F. fiskei* extract as shown in Figure 1.

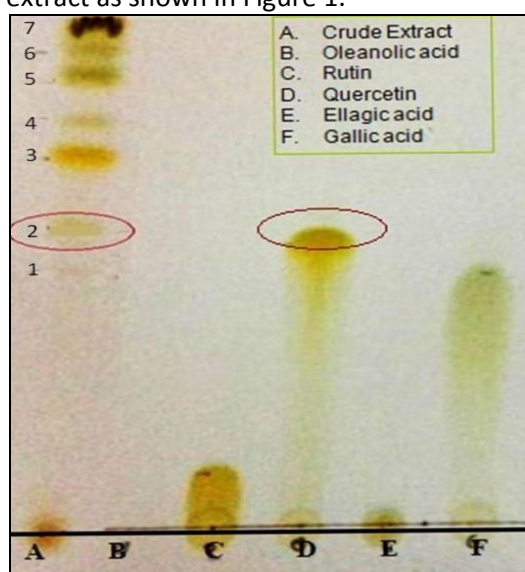


Figure 2. TLC chromatogram of the *F. fiskei* extract and the standards

One spot matches to the standard quercetin having similar Rf value of 6.3 while gallic acid (Rf value=5.5), rutin (Rf value=2.4), oleanolic acid (no spot), and ellagic acid (Rf value=1.7) do not correspond to any of the plant extract's spots, suggesting that quercetin is likely present in the *Ficus* extract. Quercetin belongs to a large class of flavonoids widely distributed in black tea, blue-green algae, broccoli, onions, red apple, and red wine. But no report has ever indicated that flavonoids are in the *Ficus* extract. Other studies revealed that Quercetin has anti-inflammatory and antioxidant actions perhaps due to its lipoxygenase inhibitory activity against pro-inflammatory compounds and prostaglandins formation [13]. Furthermore, Quercetin is a good

nitric oxide scavenger [14]. Hence, the following experiments were ascertained.

Estimation of Total Phenol Content and Flavonoid Content

A more sensitive Fast Blue BB method was employed to quantify the presence of phenolics in the crude extract. The amount was estimated from the linear equation of gallic standard curve ($y = 2.9999x + 0.1771$). For a 0.1 mg/mL of *F. fiskei* extract, a 0.019 mg/mL of gallic acid equivalent was obtained. This value is small. In contrast however, *F. fiskei* extract at 0.1 mg/mL contains 0.635 mg/mL quercetin equivalents, based on the linear equation from the quercetin standard curve ($y = 0.0635x + 0.034$) using the Aluminum Chloride method. This amount is good enough. And what do we know of flavonoids?. They are strong antioxidants that can ably arrest free radicals. Their strong proton-donating capacity and potency would have to depend on the number of hydroxyl groups present, their localization on the molecule, and electron delocalization of the aromatic nucleus which may all contribute to their high antioxidant capacity [15].

Antioxidant & Prooxidant activity

While some *Ficus* species are potent antioxidants like *F. pseudopalma* Blanco [9, 11, 12], *F. bengalensis* L. [16], *F. racemosa* L. [16], *F. glomerata* [17], *F. microcarpa* L. [18] and others like *F. pseudopalma* [12] and *F. odorata* [19] are more of prooxidants. Below highlighted specific antioxidant assays the establish the dual nature of *F. fiskei* Elm., i.e., as a pro-oxidant nor antioxidant.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay

The DPPH assay is a commonly used as the primary tool in evaluating an antioxidant activity of a plant through the latter's hydrogen donating ability. A 72.31% inhibitory activity of the extract was found at the highest concentration (5 mg/mL) and -32.79% at the lowest concentration (3.13 mg/mL). This means that *F. fiskei* is a DPPH radical scavenger at higher concentrations while it stimulates DPPH formation at lower concentrations. Median inhibitory concentration of DPPH (IC₅₀) was estimated at 2.2 mg/mL (Figure 2).

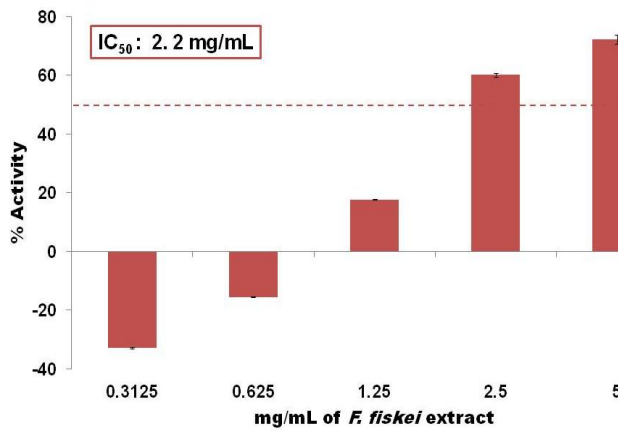


Figure 3. DPPH radical scavenging activity of different concentrations of *F. fiskei* crude extract. Values are expressed as mean±SEM (n=3), p<0.05.

FRAP (Ferric Reducing Antioxidant Power) assay

The FRAP assay is also a general test of the antioxidant capacity of plants. It is based on the ability of sample (antioxidant) to reduce Fe^{2+} ions to Fe^{3+} ions forming a blue colored complex. The higher the reducing power translates to a greater antioxidant activity. As shown in Figure 3, the reducing power of the extract was concentration-dependent, i.e., the higher the concentration of the extract corresponds to higher percentage activity in reducing ferric ions. The highest percentage of reducing power exhibited by the extract was 85.86% while the lowest percentage activity was 44.44%. Thus, the extract displays reducing activity with 50% of reducing power (RP₅₀) at 0.83 mg/mL. *F. fiskei* therefore may act as iron-reducing agent thereby preventing the excess iron participate in more free radicals formation.

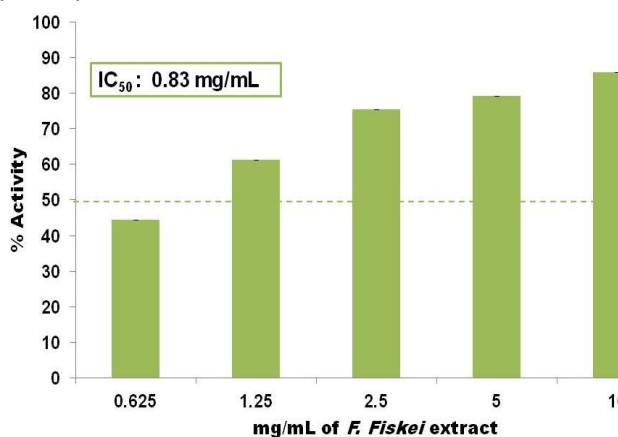


Figure 4. Ferric Reducing Antioxidant Power (FRAP) of different concentrations *F. fiskei* extract. Values are expressed as mean±SEM (n=3), p<0.05.

Superoxide radical scavenging assay

Superoxide radical ($\cdot O_2^-$) is an oxygen-centered radical that is formed from autoxidation and electron leakage from the electron transport chain onto oxygen. These radicals are potent inducer of chain reactions that can lead to production of other reactive species. As shown in Figure 5, the extract stimulates superoxide radical with SC₅₀ at 0.786 mg/mL. Hence, it was observed that a higher concentration of the extract increases the level of stimulation.

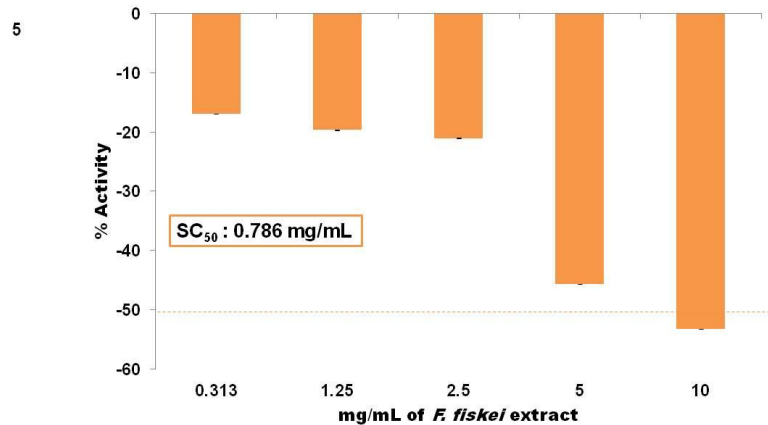


Figure 5. Superoxide stimulatory effect of different concentrations of *F. fiskei* extract. Values are expressed as mean±SEM (n=3), p<0.05.

In this case, the extract clearly displays prooxidant activity by stimulating superoxide radical production. If produced in significant amount, superoxide radical can activate white blood cells, macrophages, monocytes and neutrophils catalyzed by a membrane-bound NADPH oxidase reaction. Superoxide radical is capable then in killing foreign substances in the body, contributing in microbial and tumoricidal activity and thus lessen the virulence of the invading pathogens [20]. Two products of superoxide radicals, hydrogen peroxide and hydroxyl radicals have shown responsible for UV irradiation-induced apoptosis in HL-60 cells suggesting the indirect involvement of superoxide in the induction of tumor cell apoptosis [21].

Hydroxyl radical scavenging assay

Hydroxyl radical ($\cdot OH$) is another oxygen-centered radical that is produced through exposure to γ rays or reaction between hydrogen peroxide (H_2O_2) and superoxide radicals. The amount of $\cdot OH$ that the plant extract can inhibit was then examined. Using this assay, the $\cdot OH$ were produced by the reacting H_2O_2 with EDTA-bound Fe^{+2} . The data showed that the ethanolic leaf extract of *F. fiskei* stimulated the formation of $\cdot OH$ in a concentration-dependent manner. The median



stimulatory concentration (SC_{50}) was estimated at 0.056 mg/mL (Figure 6).

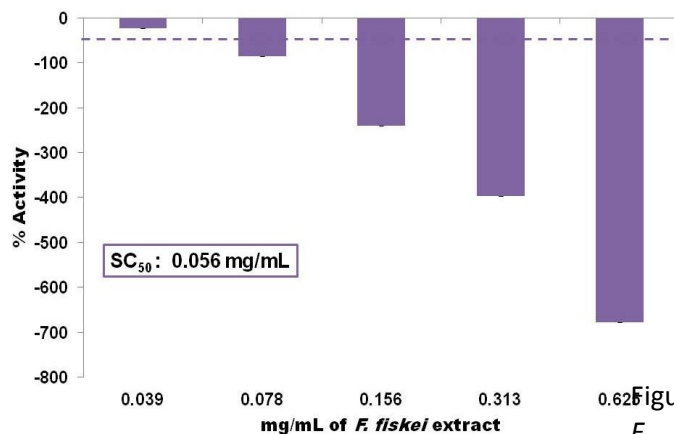


Figure 6. Hydroxyl stimulatory activity of different concentrations of *Ficus fiskei* extract. Values are expressed as mean \pm SEM (n=3), p<0.05.

It is well-known that $\cdot\text{OH}$ are the most reactive and deleterious among all free radicals that may cause oxidative stress. The other school of thought however said that $\cdot\text{OH}$ can be beneficial in cancer treatment. Cancer cells grow best in a less oxygenated environment thus having contact of these cells to oxygen-centered radicals would lead to its growth inhibition [22]. These radicals were found in blood as a natural defense against pathogens. Macrophages and granulocytes discharge said radicals in order to fight foreign bodies that may cause infections [23].

Nitric oxide radical scavenging assay

Nitric oxide radical ($\cdot\text{NO}$) is a radical that can react with oxygen to form nitrogen dioxide, which is another reactive nitrogen species. It can also react with superoxide radicals to form peroxynitrite which is an oxidizing agent in the body [20]. The $\cdot\text{NO}$ assay was carried out to evaluate the scavenging activity of the ethanolic extract of *F. fiskei*. The assay was based on Griess reaction wherein, the nitrite ions produced reacts with sulfanilamide and NED to produce a pink-colored solution. Figure 6 shows the inhibition activity of the extract against $\cdot\text{NO}$ radical.

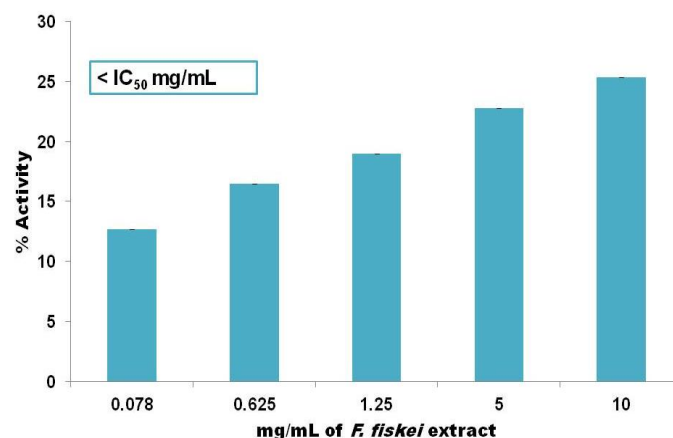


Figure 7. Inhibitory effect of different concentrations of *F. fiskei* extract against nitric oxide. Values are expressed as mean \pm SEM (n=3), p<0.05.

The crude ethanolic leaf extract of *F. fiskei* showed a steady increase on inhibiting $\cdot\text{NO}$ as the concentration of extract increased. The $IC_{50} > 10$ mg/mL. $\cdot\text{NO}$ is a physiologically active species that acts with other radicals. $\cdot\text{NO}$ is a potent inducer of cell damage that can lead to inflammation, cancer, and cardiovascular diseases [24]. Inhibiting $\cdot\text{NO}$ promotes vasoconstriction, heat retention, reduction of excessive blood loss, and prevention of hypotension [25].

On the other side, phenolics and flavonoids can act also as prooxidants in systems with redox-active metals in the presence of O_2 [26,27]. According to studies, the flavonoid-rich *Rabdosiarubescens* and phenolics such as ortho-dihydroxycoumarins derivatives, cinnamic acid derivatives and 1,2-pyrones can induce ROS production, cell differentiation, and apoptosis in U-937 cells [28,29]. If this is so, it becomes imperative to isolate and purify the specific compounds in *F. fiskei* that behave as an antioxidant or prooxidant at a given specific concentration and experimental condition. A win-win situation, we can establish compounds or drug formulations that may be developed to act separately as a prooxidant or prooxidant.

Conclusion

This study establishes the potency of ethanolic extract from the leaves of *Ficus fiskei* Elmer having dual behavior as an antioxidant and prooxidant depending on sample concentration. The main bioactive constituents responsible for said activities may be due to the presence of polar phytochemicals such as the phenolics and flavonoids. Moreover, this study provides referential pharmaco-botanical,



phytochemical information and medicinal value of the plant.

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References

1. Pancho J.V. Vascular Flora of Mount Makiling and Vicinity, Part 1. Kalikasan, The Philippine Journal of Biology, Supplement No. 1. 1983. New Mercury Printing Press, Quezon City, Philippines.
2. Sirisha, N., Sreenivasulu, M., Sangeeta, K., Madhusudhana Chetty, C. Antioxidant Properties of Ficus Species – A Review. International Journal of PharmTech Research 2010; 2(4): 2174-2182.
3. Ragasa C.Y, Tsai P., Shen C.C. Terpenoids and sterols from the endemic and endangered philippine trees, *Ficus pseudopalma* and *Ficus ulmifolia*. Philipp J Sci 2009; 132(2):205-209.
4. Lambert J. and Elias R. The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. Arch Biochem Biophys 2010; 501(1): 65-72.
5. Muller, F.L.; Lustgarten, M.S.; Jang, Y.; Richardson, A.; Van, R.H. Trends in oxidative aging theories. Free Radical Biology & Medicine 2007; 43(4): 477-503.
6. Alejandro, G.J. & Madulid, D. The Ficus (Moraceae) Flora of Mts. Palay-palay; Mataas na Gulod National Park (Ternate, Cavite). Acta Manilana 1999; 47(2): 15-40.
7. Elmer, A.D.E, and A.M. Leaflets of Philippine Botany 1908; 1(16), 195-196. Available from: <http://biodiversitylibrary.org/page/768011>
8. Yale University. (2011). Dried leaves of *Ficus fiskei* Elm. used for filing, a native sand paper. Available from: <http://discover.odai.yale.edu/ydc/Record/2237049>
9. Santiago, L. and Valerio, V. Assessment of antioxidant activities of crude ethanolic leaf extract of *Ficus pseudopalma* Blanco (Moraceae). International Journal of Pharmaceutical Frontier Research 2013; 3(1): 1-11.
10. de Vera, Estela P., Santiago, L. Toxicity profiling and assessment of in vitro antimicrobial activity of *Diospyros philippinensis* A.D.C. (Ebnaceae) methanolic leaf extract. World Journal of Pharmaceutical Research 2014; 3(4): 126-149.
11. Bueno, P., Buno, C., Santos, D. and Santiago, L. Antioxidant activity of *Ficus pseudopalma* Blanco and its cytotoxic effect on hepatocellular carcinoma and peripheral blood mononuclear cells. Curr Res Bio Pharm Sci 2013; 2(2): 14-21.
12. Santiago, L. and Mayor, A. Lupeol: An antioxidant triterpene in *Ficus pseudopalma* Blanco (Moraceae). Asian Pacific Journal of Tropical Biomedicine 2014; 4(2): 109-118
13. Green, R.J. Natural Therapies for Emphysema and COPD: Relief and healing for chronic pulmonary disorders 2007; Inner Traditions/Bear&Co.
14. Alinezhad, H., Baharfar, R., Zare, M., Azimi, R., Nabavi, S., & Nabavi, S. Biochemical activities of acetone extracts of *Hyssopus angustifolius*. Acta Poloniae Pharmaceutica – Drug Research 2012; 69(4): 617-622.
15. Žilić, S., Hadži-Tašković, Šukalović, V., Dodig, D., Maksimović, V., Maksimović, M., & Basić, Z. Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. Journal of Cereal Science 2011; 54(3): 417-424.
16. Manian, R., Anusuya, N., Siddhuraju P. and Manian S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. Food Chem 2008; 107(3):1000-1007.
17. Uttara J. and Mohini U., Evaluation of Antioxidant Activity of Aqueous Extract Bark of *Ficus glomerata*, Res J Phar Tech 2008; 1(4):537-538.
18. Ao, C., Li, A., Elzaawely, A.A., Xuan, T.D., and Tawata, S. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. Fil. Extract. Food Control 2008; 19(10): 940-948.
19. Santiago, L. and Mayor, A.B. Prooxidant Effect of Crude Ethanolic Leaf Extract of *Ficus odorata* Blanco Merr. in vitro: Its medical significance. International Journal of Biological Food, Veterinary and Agricultural Engineering 2014; 8(1): 53-60.
20. Gropper, S., Smith, J., and Groff, J. Advance Nutrition and Human Metabolism. 5th edition. Cengage Learning 200; Wadsworth, USA.
21. Gorman, A., McGowan, A. and Cotter, T.G. Role of peroxide and superoxide anion during tumor cell apoptosis. FEBS Letter 1997; 404(1): 27-33.
22. Wong CC., Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the Ferric Reducing Antioxidant Power Assay. Food Chem 2006; 97(4): 705-711.
23. Jakovljevic, V. L.; Cubrilo, D.; Zivkovic, V.; Djordjevic, D., and Djuric, D. Exercise and Oxidative Stress, Oxidative Stress. Environmental induction and dietary antioxidants. 2012. Available from <http://www.intechopen.com/books/oxidative-stress-environmental-induction-and-dietary-antioxidants/exercise-and-oxidative-stress>.
24. Murrell GA, Dolan MM, Jang D, Szabo C, Warren RF, Hannafin JA. Nitric oxide: an important articular free radical. J Bone Joint Surg Am 1996; 78(50): 265-274.
25. Moncada, S.; Higgs, E.A. The discovery of nitric oxide and its role in vascular biology. British Journal of Pharmacology 2006; 147(S1):193-201.
26. Galati, G., and O'Brien, P. Potential toxicity of flavonoids and other dietary phenolics: significance for their



chemopreventive and anticancer properties. *Free Radical Biology and Medicine* 2004; 37(3): 287–303.

27. Iwasaki, Y., Hirasawa, T., Maruyama, Y., Ishii, Y., Ito, R., Saito, K., et. al. Effect of interaction between phenolic compounds and copper ion on antioxidant and pro-oxidant activities. *Toxicology in Vitro* 2011; 25(7): 1320–1327.

28. Bai, N., He, K., Zhou, Z., Lai, C.-S., Zhang, L., Quan, Z., et. al. Flavonoids from *Rabdosia rubescens* exert anti-inflammatory and growth inhibitory effect against human leukemia HL-60 cells. *Food Chemistry* 2010; 122(3): 831–83.

29. Riveiro, M.E., Moglioni, A., Vazquez R. et al. Structural insights into hydroxycoumarin-induced apoptosis in U-397 cells. *Bioorganic and Medicinal Chemistry* 2008; 16(5): 2665-2675.

30. Abdel-Hameed, E.-S.S. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. *Food Chemistry* 2009; 114(4): 1271-1277.

31. Merrill, E.D. *An Enumeration of Philippine Flowering Plants Vol. 2* (2013). Bureau of Printing, Manila.

32. Verma, A.R., Vijayakumar, M., Rao, C.V., and Mathela, C.S. In vitro and in vivo antioxidant properties and DNA damage protective activity of green fruit of *Ficus glomerata*. *Food and Chemical Toxicology* 2010; 48(2): 704-709.

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.