

Nutritional composition analysis of *Ficus odorata* (Blanco) Merr.: a road to its prebiotic potential

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Ficus odorata (Blanco) Merr., is an indigenous ethnomedicinal plant widely cultivated in Luzon and commonly used by the Aetas of Bataan for treating several ailments. There are no known reports as to its nutritional composition hence, this study. Thus, the study aimed to unravel the nutritional contents of *F. odorata* and relate it to the possible application on various medications for certain diseases and as a candidate for being a prebiotic agent. Results show that the plant contains high carbohydrates (45.7%) and dietary fibers (36.1%) with sufficient amount of proteins (15.2%) and less total fat (4.7%) content. Lastly, micronutrient analysis revealed that the plant is rich in calcium (3.35%) followed by potassium (1.38%) and with trace amounts of sodium and zinc. *Ficus odorata* is a fiber-rich food ingredient that can provide a wide range of health benefits. Moreover, it can be used as a prebiotic agent that can ably support the growth of probiotics. Therefore, *F. odorata* leaf powder may be a potential functional food component as prebiotics for probiotics.

Keywords: *Ficus odorata*, endemic, nutrition, prebiotics, dietary fibers

INTRODUCTION

Ficus odorata (Blanco) Merr., commonly known as *Pakiling* or *Is-is*, is an indigenous ethnomedicinal plant of the mulberry family (Moraceae). It is widely cultivated in Tayabas, Zambales, Pampanga, Laguna, and Bataan and extensively used by the Aetas of Bataan, Philippines for several ailments including diabetes, cancer, tumor, allergy, asthma, and diarrhea [1]. Though the plant is widespread for having many medicinal uses, scientific studies

scarcely support these claims. Which is why, investigations were conducted in order to provide a more conclusive explanation as to how the plant provides major health benefits.

A previous study validated the plant's hypoglycemic activity on streptozotocin-induced diabetes rat model wherein the dichloromethane (DCM) fraction of *F. odorata*, both 50 mg/kg and 200 mg/kg concentration *per orem*, produced a significant decrease in the mean fasting blood sugar levels of diabetic rats after a 14-day treatment and is safe at 2000 mg/kg dose [2]. In another study, ethanolic crude

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leaf extract of *F. odorata* was established to have a pro-oxidant activity towards a group of physiologically important free radicals such as superoxide anion and hydroxyl free radicals and hydrogen peroxide [3]. A recent study substantiates its anticancer potential. The plant was shown to be cytotoxic and genotoxic on human hepatocellular carcinoma cell lines (HepG2) *in vitro* wherein *F. odorata* inhibited the growth in a concentration-dependent manner ($IC_{50} = 25 \mu\text{g/mL}$) and likewise induced DNA damage even at $20 \mu\text{g/mL}$ concentration [4]. The chemical constituents of the *F. odorata* has been previously identified through 1D and 2D NMR spectroscopy and was shown to have β -sitosteryl-3 β -glucopyranoside-6-O-palmitate, squalene, lutein, α -amyrin acetate, lupeol acetate, and β -carotene [5]. But no studies so far dealt on the nutritional composition of *F. odorata* that could probably provide insight and understanding on why its dietary nutrients can be relevant.

MATERIALS AND METHODS

Assessment of the nutritional content of F. odorata

Plant collection and processing. Fresh leaves of *F. odorata* were collected from Barangay Igbac, Buhi, Camarines Sur in Bicol, Philippines and was authenticated by the Botany Division of the Philippine National Museum. The leaves were air-dried and were ground using Wiley-Mill grinder and the powdered leaves were stored in a tightly sealed plastic container [3, 4].

Chemical analysis. The powdered leaves of *F. odorata* were sent to the Food Analytical Services Laboratory of the Department of Science and Technology-Food and Nutrition Research Institute (DOST-FNRI), Philippines to analyze and determine its nutritional value as summarized in Table 1.

Table 1. Chemical analysis performed to the powdered leaves of *F. odorata* for the assessment of its nutritional content

| Analyte | Method |
|--------------------------------|--------------------------------------|
| Total Fat | Acid Hydrolysis |
| Ash | Gravimetric |
| Moisture | Air/Oven Vacuum Oven |
| Dietary Fiber | Enzymatic, AOAC |
| Total Carbohydrates and Energy | Computation from proximate analyses |
| Crude Protein | Kjeldahl method |
| Sodium | Atomic Absorption Spectroscopy (AAS) |
| Potassium | AAS |
| Calcium | AAS |
| Zinc | AAS |

Evaluation of the antibacterial activity

Plant preparation and extraction. One (1) kilogram of the powdered leaves of *F. odorata* was soaked in 2.5 L ethanol (95%) for 5 days with extract collection every after 24 h. The collected leaf extracts was concentrated using a rotary evaporator (Eyela, USA) at 40°C . The concentrated crude ethanolic leaf extract of *F. odorata* was stored in an amber bottle and was placed inside a refrigerator at 4°C .

Disc-diffusion method

Preparation of bacterial inoculums and discs. Three Gram-positive and Gram-negative strains of bacteria maintained in Mueller Hinton (MH) agar were used in the study namely, *Staphylococcus epidermidis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. A loopful of each strain of bacteria was inoculated on MH broth and was incubated for 24 h at 37°C . Then, the turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard, that is approximately equivalent to 1.5×10^8 CFU/mL. This was used for the standardization of every antibacterial assay.

Whatman filter paper No. 1 discs of 6 mm diameter were sterilized using hot air oven at 160°C for 1 h. Then 20 µL of the test extract containing 2 mg/mL was introduced on the sterile paper discs, and the solvents were allowed to evaporate on a stream of air. The solvents used for dissolving the extracts served as negative controls, while reference antibiotics cephalothin (C; 30 µg), vancomycin (V; 30 µg), amikacin (A; 30 µg) and bacitracin (B; 10 U) were used as positive controls.

Antibacterial agar disc diffusion assay: Kirby-Bauer method. An aliquot of 0.5 mL bacterial suspension containing 1.5×10^8 CFU/mL was transferred on the surface of the MHA plates and was spread all over three times by rotating the plate 60° after each streaking using a sterile cotton applicator. Subsequently, filter paper discs with the crude extract of *F. odorata*, negative control, and reference antibiotic discs were placed on the surface of the agar at equidistant points using sterile forceps. The plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition to the nearest millimeter around the disc using a Vernier caliper. The tests were carried out in triplicate, and results were recorded as mean \pm SD.

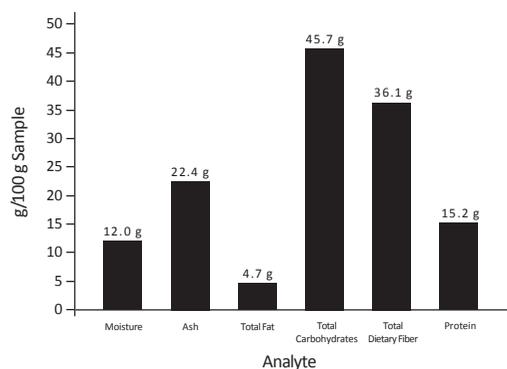


Figure 1. Proximate analysis of the major nutritional content of *F. odorata*.

RESULTS AND DISCUSSION

Phytochemicals had contributed much to the recorded medicinal applications in every plant. Owing to a wide-range of benefits derived from indigenous plants, they become significant sources of dietary food supplements. Nowadays, consumption of fiber-rich plant sources is considered as the most leading functional dietary trends in the United States [6].

Figure 1 shows the total estimates for the major dietary components of *F. odorata* leaf. The plant contains 45.7% total carbohydrates and 36.1% total dietary fibers which the body may use readily as energy sources. Intake of dietary fibers (soluble and insoluble) lend several health benefits including risk-reduction for the development of coronary heart disease, stroke, hypertension, diabetes, obesity and gastrointestinal (GI) diseases [7]. In the Philippines, coronary heart disease (13.73%) is the most prevalent cause of death followed by stroke (9.55%) and hypertension (8.30%) [8]. Their occurrence is usually related to the lifestyle practices such as dietary patterns, physical activity and cigarette abuse [9, 10]. And that food consumption high in dietary fibers could reduce their prevalence [6, 7]. A study had noted about 14% decrease in risk of all coronary events and 27% decrease in risk of

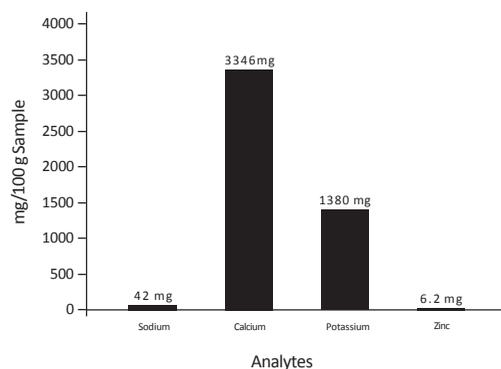


Figure 2. Proximate analysis of the micronutrients in *F. odorata*.

coronary deaths during a 6–10-year follow-up investigation involving a 10 g/day increment of energy-adjusted [11] and measurement error-corrected total dietary fiber consumption [7].

Moreover, inclusion of dietary fibers in the diet may also reduce the risk for diabetes mellitus. Fiber-rich foods are less energy-dense and larger in volume, which limits the spontaneous intake of energy and bring a feeling of satiety [12]. Furthermore, it is also considered to lower serum glucose level at 30–50 g fiber per day and fiber supplements at 10–29 g fibers per day may help in controlling sudden changes in the glycemic level [13]. These findings may also be related to the hypoglycemic activity of *F. odorata* towards streptozotocin-induced diabetes in Sprague-Dawley rats [2]. In this particular study, the DCM fraction of the plant (50 mg/kg and 200 mg/kg) exhibited a significant decrease in the mean fasting blood sugar levels of diabetic rats after the 14-day treatment.

The plant contains also 15.2% total proteins. A diet containing wide variety of fiber-containing foods is also usually richer in micronutrients.

Figure 2 shows micronutrients are present in *F. odorata* leaf wherein calcium (3.35%) is the highest followed by potassium (1.38%) with trace amounts of sodium and zinc. These findings strongly suggest that its leaf is a highly nutritious food source for every undernourished and indigent Filipino.

Not only is *F. odorata* a good functional food ingredient in helping curb different lifestyle diseases but may find its way in the probiotic nutraceuticals inasmuch as it contains high amounts of carbohydrates, dietary fibers, proteins and micronutrients. As these molecules can serve as prebiotic food sources for probiotic bacteria (lactic acid and *Bifidobacteria*) they support synbiotic connection of gut microbiome. In diet, prebiotics are typically insoluble fiber compounds that enter the GI tract that induce the growth and/or activity of the gut microbiota.

To support the claim, antibacterial assay was performed on the freshly prepared *F. odorata*. Results showed no antibacterial activity against several standard ATCC strains such as *Staphylococcus epidermidis*, *Bacillus cereus*,

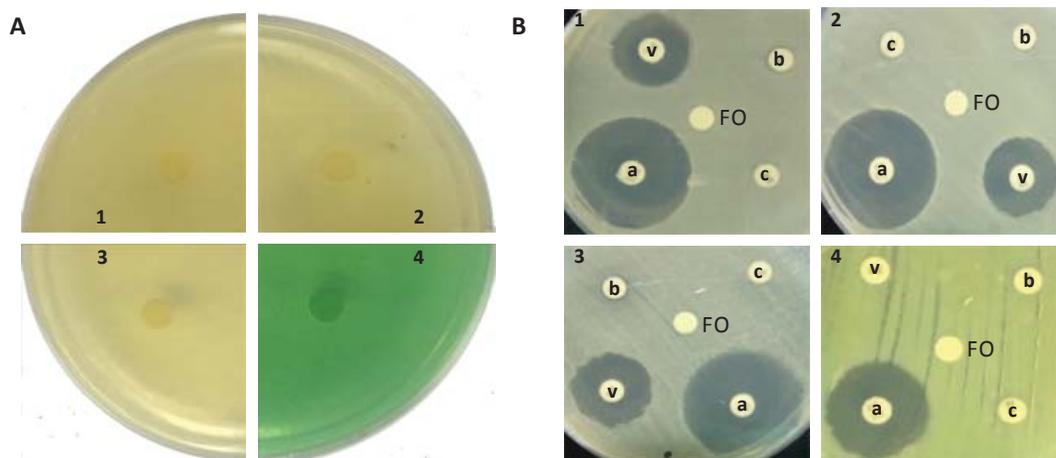


Figure 3. Disc diffusion assay. (A) No zone of inhibition was observed after a 24-h incubation of the crude ethanolic leaf extract of *F. odorata* with (1) *B. cereus*, (2) *K. pneumoniae*, (3) *S. epidermidis*, and (4) *P. aeruginosa*. (B) Zone of inhibition by standard antibiotics (a) Amikacin (b) Bacitracin (c) Cephalothin, and (v) Vancomycin in contrast with the crude ethanolic leaf extract of *F. odorata*

Pseudomonas aeruginosa, and *Klebsiella pneumoniae* (Fig. 3). Results showed that *F. odorata* displayed no antibacterial activity with *standard test microorganisms*. However, most studies on other *Ficus* species on various parts of the plants extracted with different solvents including *F. benghalensis*, *F. racemosa*, *F. auriculata*, *F. religiosa*, *F. glomerata*, *F. nitida*, *F. retusa*, *F. asperifolia*, *F. exasperate*, *F. carica*, *F. lyrata*, *F. deltoidea*, *F. palmate*, and *F. capensis* revealed moderate to high antibacterial activity towards various strains [14].

Conversely, latest experiments demonstrated that the leaf extract of *F. odorata* can stimulate and sustain the growth of lactic acid bacteria in enrichment and cultivation MRS (de Man, Rogosa and Sharpe) agar and broth indicating that the extract has an important functional food ingredient that works as a prebiotic capable of supporting proliferation of probiotic bacteria [15]. An elaborate work on this is in progress.

CONCLUSION

Ficus odorata Blanco is a potential functional food component with high dietary fiber, carbohydrates, proteins and mineral relevant in the formulation of probiotic nutraceuticals.

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REFERENCES

- [1] Herrera AA, Ramos JB, Ibulan LA. Teratogenic activity of *Ficus odorata* (Blanco) Merr. and *Baccaureate tandra* (Baill.) Müll in mice embryos. *Phil Agri Sci.* **2010**; 93:255–262.
- [2] Degollado JV, Yolo RT, Santiago LA. Hypoglycemic effect and *in vitro* antioxidant activity of the dichloromethane fraction from the leaves of *Ficus odorata* (Blanco) Merr. (Moraceae). *Int. J. Res. Dev. Phar. Life Sci* **2014**; 3(5):1163–1173.
- [3] Santiago LA, Mayor ABR. Prooxidant Effect of the Crude Ethanol Leaf Extract of *Ficus odorata* (Blanco) Merr *in vitro*: Its Medical Significance. *Int. J. Biol. Life Sci. Eng.* **2014**; 8(1):19–26.
- [4] Santiago LA, Dayrit KC, Correa PCB, Aguila ACM, De Leon MRM, Mayor ABR. Cytotoxicity and genotoxic activities of the crude ethanol leaf extract of *Ficus odorata* (Blanco) Merr. against human hepatocellular carcinoma. *J. Pharmac. Phytochem.* **2014**; 3(3):33–38.
- [5] Tsai PW, De Castro-Cruz KA, Shen CC, Chiou CT, Ragasa CY. Chemical Constituents of *Ficus odorata*. *Pharma. Chem. J.* **2012**; 46(4):225–227.
- [6] Slavin JL. Position of the American Dietetic Association: health implications of dietary fiber. *J. Am. Diet. Assoc.* **2008**; 108(10):1716–1731.
- [7] Anderson JW, Baird P, Davis RH, Ferreri S, Kundtson M, Koraym A, Waters V, Williams CL. Health Benefits of Dietary Fiber. *Nutrition Reviews* **2009**; 67(4):188–205.
- [8] World Health Organization. *World Health Rankings, Philippines 2011*. (<http://www.worldlifeexpectancy.com/country-health-profile/philippines>).
- [9] Stampfer MJ, Hu FB, Manson JE, Rimm EB, Willett WC. Primary prevention of coronary heart disease in women through diet and lifestyle. *New Engl. J. Med.* **2000**; 343:16–22.
- [10] Kris-Etherton PM, Etherton TD, Carlson J, Gardner C. Recent discoveries in inclusive food-based approaches and dietary patterns for reduction in risk for cardiovascular disease. *Curr. Opin. Lipidol.* **2002**; 13:397–407.
- [11] Pereira MA, Jacobs Jr. DR, Pins JJ, *et al.* Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. *Am. J. Clin. Nutr.* **2002**; 75:848–855.
- [12] Giacco R, Rivellese AA, Lasorella G, Giacco A, Episcopo LD, Riccardi G. Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. *Diabetes Care* **2000**; 23(10):1461–1466.

- [13] Jenkins DJ, Kendal CW, Vuksan V, Vidgen E, Parker T, Faulkner D, Mehling CC, Garsetti M, Testolin G, Cunnane SC, Ryan MA, Corey PN. Soluble fiber intake at a dose approved by the US Food and Drug Administration for a claim of health benefits: serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. *Am. J. Clin. Nut.* **2002**; 75(5):834–839.
- [14] Salem MZM, Salem AZM, Camacho LM, Ali HM. Antimicrobial activities and phytochemical composition of extracts of *Ficus* species: An overview. *African J. Microbio. Res.* **2013**; 7(33):4207–4219.
- [15] Panganiban ELC, Santiago LA. Development of Probiotic Nutraceuticals: Prebiotic effect of *Ficus odorata* Blanco on the growth of *Lactobacillus salivarius* isolated from human breast milk. *Book of Abstracts of the 41st Annual Convention of the Philippine Society of Biochemistry and Molecular Biology*, p. 139. (Marco Polo Plaza Hotel, Cebu City; December 4–5, 2014).