Herbal Medicines Journal 2019; 4(4):141-56
DOI: 10.22087/herb%20med%20j.v4i4.761 **ISSN:** 2538-2144

Original Article

Survey and *In Vitro* Investigation of the Plants Used by Traditional Healers in the Treatment of Diabetes in Albay Province, the Philippines

Dennis B. Gogola[1*], Maria Luisa U. Tango[2]

[1]Faculty, Nursing Department, Bicol University Tabaco Campus, Philippines
[2]Faculty, Education Department, Bicol University Tabaco Campus, Philippines

Received: 13.07.2019; Accepted: 17.10.2019

Abstract

Background and Aim: Diabetes is a global lifestyle disease that affects the health and economy of the affected population. It causes debilitating complications, which require expensive medications whose consumption produces side effects. Likewise, this study identified the plants used for treating diabetes by traditional healers in Albay province, Philippines. We evaluated their anti-diabetic and antioxidant activities *in vitro,* and determined the presence of essential phytochemicals.

Materials and Methods: The anti-diabetic activity of the plant extracts was determined by α-glucosidase inhibition assay. The free radical scavenging activity of the plants was evaluated using DPPH (1, 1-Diphenyl-2 -picrylhydrazyl). Moreover, the determination of total phenolics, tannins, and flavonoids was performed using conventional methods.

Results: The nine plants that were used included *Annona muricata* L., *Blumea balsamifera* (L.) DC., *Moringa oleifera Lam, Cymbopogon citratus* (DC.), *Cocus nucifera* L., *Curcuma longa* L., *Momordica charantia* L., *Abelmoschus esculentus* (L.) Moench., and *Citrus microcarpa* Bunge. The inhibitory activity against α-glucosidase was actively exhibited by *B. balsamifera* (L.) DC. leaf and *C. nucifera* L. root extracts with IC50 (inhibition concentration at 50%) values of 4.97 and 13.36 μg/ml, respectively. Furthermore, the plant extracts exhibited free radical scavenging capacity that ranged from 38.88 to 90.36% at the concentration of 100 μg/mL. The *B. balsamifera* (L.) DC. exhibited the highest radical scavenging activity, with 90.36%, followed by *C. nucifera* L. with 88.57 %. Furthermore, the total phenolic, flavonoid, and tannin contents were found higher in *C. nucifera* L. with 609.5, 98.3, and 1,577.70mg GAE/g dw, respectively. Meanwhile, in *B. balsamifera* (L.) DC. there were 276.4mg GAE/gm phenolic, 611.1mg GAE/gm tannin and 63.3mg GAE/gm dw flavonoid contents, respectively.

Conclusion: The results suggest that only two out of the nine plants displayed inhibitory activity against α-glucosidase. Furthermore, they exhibited antioxidant capacity and contained essential bioactive compounds. Hence, the results provide scientific support to the potential use of these plants in the traditional alternative medicine for treating diabetes.

Keywords: Diabetes, Alpha-glucosidase inhibition, Antioxidant, *C. nucifera* L., *B. balsamifera* (L.) DC.

***Corresponding Author:** Dennis B. Gogola, Nursing Department, Bicol University Tabaco Campus, Albay, Philippines. Email: dennisgogola@yahoo.com; Tel: 0936-248-0733.

Please cite this article as: Gogola DB, Tango MLU. An *In Vitro* Investigation of the Plants Used by Traditional Healers in the Treatment of Diabetes in Albay Province, the Philippines. Herb. Med. J. 2019; 4(4):141-56.

Introduction

Diabetes mellitus (DM), which is usually referred to as 'diabetes,' is a metabolic disease characterized by high blood glucose level caused by either an insufficient amount of insulin production by the pancreas or the failure of the body to use the insulin it produces effectively. In 2015, the World Health Organization (WHO) reported that roughly 1.6 million individuals died due to diabetes (1). Diabetes is considered to be a chronic disease which is increasing in both prevalence and incidence worldwide (2). It is also regarded as one of the major causes of death (3) in many countries. Similarly, it was reported that by the year 2025, Asia would have the greatest step-up in the number of people with diabetes (4). Hence, this situation might have a significant impact on some developing countries such as the Philippines. As it was reported by the International Diabetes Federation, the prevalence rate of type 2 diabetes in the Philippnines was approximately 3.2 million people in 2014. Moreover, the cost of drugs in the Philippines is identified to be a barrier in diabetes care because of some tax charges that made it costly (5).

With this, it is undeniable that the health systems worldwide are undergoing rising levels of chronic diseases and consequent increase in health care costs. Thus, both the patients and providers of health care urge on the restoration of health care services. They emphasize on individualized, person-centered care (6) that invloves enlarging, advocating the access, and utilizing Traditional and Complementary Medicine (T&CM) products, practices, and practitioners. WHO announced that Traditional Medicine (TM) in many developing countries has a significant role in fulfilling the essential health care requirements of people. Specific models of TM have long been used. A remarkable instance of resorting to TM is the utilization of herbal medicines as a remedy for preventing and treating various ailments.

Consequently, herbal plants have become the central subject of research that would establish their potential therapeutic properties in the prevention and treatment of various human ailments such as diabetes. Akin to this research undertaking, the researchers identified the commonly used plants for diabetes suggested by traditional healers in selected rural areas in the Province of Albay, Philippines. It includes *A. muricata* L., *B. balsamifera* (L.) DC., *A. esculentus* (L.) Moench., *C. longa* L., *C. citratus* (DC.), *M. charantia* L., *C. microcarpa* Bunge, *C. nucifera* L., and *M. oleifera Lam*.

Some of the identified plants used for diabetes were classified to be trees such as the *A. muricata* L., *M. oleifera Lam*, *C. nucifera* L. and *B. balsamifera* (L.) DC. *A. muricata* L. is a fruit-bearing tree which is locally called as "Guyabano'. It has a long history of traditional use. Almost all parts of this tree have been used as traditional remedies against a variety of abnormal human conditions (7). *M. oleifera Lam* is widely known as 'Malunggay' in the Philippines. It is a useful backyard plant in the country. As a small fast-growing evergreen tree, it is drought resistant that makes it available throughout the year. It has some medicinal properties for which its young leaves, flowers, seeds, and tender pods are widely consumed (8). Moreover, *C. nucifera* L. or coconut tree, is a branchless palm tree which is native and is commonly known as 'Niyog' in the Philippines. All parts of the coconut tree are considered to have both economic value and medicinal significance. Some of its parts have been used in Siddha system of medicine to treat various health conditions (9). Furthermore, *B. balsamifera* (L.) DC. is a small tree which is locally known as 'Sambong'. It has been utilized as medicine not only in the Philippines but also in other Southeast Asian countries. Its whole plant and its crude extracts were found to exhibit multiple biological activities (10).

On the other hand, other plants were classified as vegetable plants, and some were used as spices in culinary. *M. charantia* L., locally called 'Ampalaya' in the Philippines, has a long history of being used for certain purposes, *e.g.* as food and medicine (11). *A. esculentus* (L.) Moench., commonly known as 'Okra', is a locally cultivated flowering plant which is used in the traditional systems of medicine such as Ayurveda, Siddha, and Unani (12), whereas, *C. citratus* (DC.) is a native herb cultivated in tropical and subtropical countries like the Philippines, and its local name is 'Tanglad.' Moreover, this plant has a long history of extensive therapeutic uses in traditional and Ayurvedic medicine in many countries (13). *C. longa* L. is commonly known as 'Turmeric'. Its local name is 'Luyang dilaw' in the Philippines. It is a significant spice crop grown abundantly in India and other tropical countries (14), which is widely used as a therapeutic remedy in Ayurvedic, Unani, and Siddha Herbal System (15). Furthermore, *C. microcarpa* Bunge, commonly known as 'Calamansi' in the Philippines, is commonly consumed as a drink in Asia which could be added to either black tea or plain water to be consumed in several forms such as food (pickle), flavoring, and a deodorant (16). It also contains polyphenols that enhance several biological properties (17).

This study aimed at investigating the plants used for diabetes by traditional healers in the Province of Albay, Philippines, by subjecting these plants to phytochemical screening, antioxidant activity testing, and determining their hypoglycemic activity *in vitro*. The results of this investigation might serve as a substantial basis for conducting further explorations of these plants. Nevertheless, the research findings would further establish the scientific basis for utilizing the indigenous knowledge, practices, and the use of traditional herbal plants as sources of alternative health care remedies for diabetes.

Materials and Methods

Plants Used for the Treatment of Diabetes by Traditional Healers

A descriptive survey was carried out to establish information about the plants used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines. A purposive sampling was used to identify the well-known traditional healers in these areas. Key informant interviews (KII) were conducted among the prominent traditional healers in the province. The interviews were carried out at the barangays of selected municipalities and in a city of the first and second congressional districts of Albay. There were two (2) barangays from Rapu-Rapu, one (1) from Sto. Domingo, two (2) from Bacacay, one (1) from Malilipot, two (2) from Tabaco City and finally one (1) barangay from Tiwi, Albay. There were about ten (10) traditional healers who were interviewed, and with their consent, they were asked about the plants used for diabetes. An interview, guided with a set of questionnaire, was conducted to answer the questions concerning the prescribed medicinal plant/s in the treatment of diabetes, including the plants' local name, plant part/s used, and methods of preparation and utilization.

Plant Materials Collection and Taxonomic Identification

The identified plants and its materials/parts used in the treatment of diabetes were similarly obtained from the nearby places within the traditional healers' location, and with their assistance that ensures the appropriateness of plants collected. However, some plants that are not available within their respective areas were collected at some places in the Province of Albay that were later verified with individual traditional healers. After the accomplishment of plant collection, the required sections of the plant samples were washed under running tap water and then were thoroughly dried with a clean cloth. A few samples of the plants' specimen sent to the Botany Department of the National Museum, Philippines, that verifies and provides the scientific names, including its genus or family. Moreover, every plant specimen was identified according to the description of the species.

'Guyabano,' scientifically called *A. muricata* L., belongs to the family Annonaceae. Moghadamtousi *et al.* described it as a terrestrial plant and an erect tree that is 5–8 meters in height. Its leaves comprise an open, roundish canopy which is large, glossy, and dark green.

B. balsamifera (L.) DC. is a half-woody perennial plant or subshrub which is categorized as a member of the Asteraceae family. According to Pang *et al.*, its height reaches up to 3 meters tall. Moreover, the shape of its leaves is either wide oval or oblong-lanceolate at the base, which is 22–25 cm in length and 8–10 cm in width.

M. oleifera Lam is referred to as horse-radish tree or Ben oil tree in English, and belongs to the moringaceae family. Vinoth *et al*. indicated that it is a small fast-growing evergreen tree with its height reaching about 10–12 m, with an open crown of drooping fragile branches. Moreover, it has feathery foliage with tripinnate leaves and thick corky with whitish bark.

C. citratus (DC) Stapf., which is widely known as lemongrass, belongs to the family of Poaceae/ Gramineae. There is a small bunch of perennial C4 grasses with multiple stiff stems arising from a short, rhizomatous rootstock, that grows in dense clumps. Typically, it can reach up to 1.8 m in height and about 1.2 m in width, with a short rhizome (18).

Leprosy gourd or bitter gourd, with the scientific name *M. charantia* L., is a flowering vine in the family Cucurbitaceae. Its fruit, which is ovoid, ellipsoid, or spindle in shape, usually ridged or warty and indehiscent varies in size (19).

C. nucifera L. is a branchless palm tree belonging to the Palmae family with a smooth, columnar, light grey-brown trunk on the top of which there is a terminal crown of leaves. It might reach a height of 24-30 m (giant coconut). Its trunk is thin and to a small degree swollen at the base, usually straight up but maybe leaning or curved. The pinnate leaves are feather-shaped with 4-7 m long and 1-1.5 m wide at the broadest part. Moreover, the leaf stalks are 1-2 cm in length and thornless (20).

A. esculentus (L.) Moench is one of the most familiar and utilized species of the family Malvaceae. It is an erect, coarsely haired herb with cordate, 3 to 5-lobed, and with scabrous leaves. The flowers are large, yellow with a crimson center. The capsules are ribbed, and the seeds are round (21).

C. longa L. belongs to the family *Zingiberaceae* that includes more than 80 species of rhizomatous perennial herbs. Ashraf *et al.* maintained that it could be considered a perennial herb with pulpy, highly branched, yellow-to-orange, cylindrical, aromatic rhizomes, and tuberous roots that grow to about 2 feet in length.

C. microcarpa Bunge is a Citrus fruit categorized as a member of the genus Citrus of the family Rutaceae. Its fruit is like a small round lime that has an average diameter of up to 4.5 cm. Its color resembles the orange color of a tangerine with a very thin peel that has a green or orange color (22).

Preparation of the Extracts

The extraction method of the identified plants were considered the local preparation practiced by traditional healers, but with some modifications regarding the number of plant materials and the volume of water as the solvent to meet the required amount of plant extracts needed for various *in vitro* tests. Of the nine (9) plants, there were about six (6) plant materials extracted by boiling method, namely *A. muricata* L., *B. balsamifera* (L.) DC., *M. oleifera Lam*, *C. citratus* (DC) Stapf., *C. nucifera* L., and *C. longa* L.; while *M. charantia* L. was conducted by blanching; the *A. esculentus* (L.) Moench was carried out by water soaking; and *C. microcarpa* Bunge was performed by juicing the fruit as shown in Table 1. The researchers used 250g of each plant material and 750 ml of tap water in every extraction procedure for boiling, blanching, and soaking. The boiling and blanching procedures lasted for about 30 minutes, and the soaking method was done overnight; while juicing was carried out a few hours before the filtration. The plant parts that were boiled in water produced an extract in amber appearance, whereas the extract of plant material prepared by blanching appeared to be in a slightly lighter green color. Moreover, the extracts of plant materials that were produced by soaking and by juicing appeared to be in clear slimy liquid and yellow-orange color, respectively. Subsequently, the crude aqueous extract was filtered and freeze-dried in order to produce the lyophilized crude aqueous extracts.

Determination of Total Phenolic Content

The total phenolic content in the plant extract was assessed spectrophotometrically (UH5300) by the use of the Folin-Ciocalteu method (23). Gallic acid was applied as the standard. An aliquot of the crude extract and standard was added to Folin Ciocalteau reagent and 7.5% sodium carbonate. Absorbance was read at 765nm. The content of phenolic compounds was determined from the standard curve and was stated as gallic acid equivalent (GAE) in mg per gram dry weight (mg/g dw of the extracted compound). All the tests were analyzed in triplicates.

Determination of Total Tannin Content

The tannin content of the extract of nine plants was assessed using the Folin - Ciocalteu method (24). Tannic acid was utilized as the standard. An aliquot of the crude extract and the standard was added to distilled water, Folin- ciocalteau reagent, and 35% sodium carbonate. The blended material was shaken and maintained at room temperature, and absorbance was read at 725 nm. All the samples were analyzed in triplicates.

Determination of Total Flavonoid Content

The flavonoid content of the extract of nine plants was determined using aluminum chloride colorimetric assay (25, 26). Quercetin was used as the standard. An aliquot of the crude extracts and standard was added to distilled water, sodium nitrite, and aluminum chloride. The mixtures were incubated and then added to NaOH and was finally mixed. The mixtures were allowed to become cool, and the absorbance was measured at 510 nm. The total flavonoid content was identified from the calibration curve and was expressed as mg quercetin equivalent per g of dry weight. All the samples were analyzed in triplicates.

Determination of Antioxidant Activity by DPPH-scavenging Assay

The free radical scavenging capacity of the extracts of nine plants and standard solution (ascorbic acid) were examined by the use of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (26). The assay mixture encompassed 2 ml of 1.0 mmol/L DPPH radical solution that was prepared in methanol and 1 ml of standard or extract solution of distinct concentrations (5-100mcg/mL). The solution was quickly blended and incubated in the dark at 37 °C for 30 minutes. The absorbance of every solution was calculated at 517 nm against a blank by spectrophotometer (UH5300). Ascorbic acid was utilized as the reference standard, and the reaction without the samples was used as the control. All the determinations were carried out in triplicates. The percentage of radical scavenging (5%) was calculated by the following formula:

$$\%\text{ Free radical scavenging activity} = \frac{A_c - A_8}{A_c} \times 100$$

Where A_c = Absorbance of control at 517 nm; A_8 = Absorbance of plant sample/extract.

The concentration of the sample that was needed to scavenge 50% of the DPPH free radical (IC50) was calculated from the curve of percent inhibitions plotted against the respective concentration.

Alpha-Glucosidase Inhibition Assay

The test was performed based on the method described by other researchers. Stock substrate solution (1.86 mM p- nitrophenol-α-D-glucopyranoside in phosphate buffer) and stock alpha-glucosidase solution (120 mU/mL in phosphate buffer) were prepared. Samples were dissolved using dimethylsulfoxide (DMSO), and then were homogenized using vortex mixer. Subsequently, they were centrifuged. One hundred ninety (190) microliters of 50 mM sodium phosphate buffer solution (containing 100 mM NaCl at pH 6.8), 10 µL of sample (final well concentration of 1 µg/ml) and 50 µL of the enzyme solution (final well concentration of 20 mU/mL) were placed in each well of 96-Well Microtiter plate. After incubating for 10 minutes at 37^{o}C, 50 µL of the substrate (the final adequate concentration of 1.86 mM) was added to start the reaction (making the total volume of 300 µL per well). The assay relies on the liberation of p-nitrophenol by enzymatic hydrolysis of the substrate, and this is correlated with the activity of the sample when compared with the negative control. The absorbance of the liberated p-nitrophenol was measured at 405nm every 30 seconds for 30 minutes using Multiskan Go® UV/VIS Spectrophotometer. The 200 microliters of acarbose (effective good concentration of 1,000 ppm in 3.33% DMSO in a buffer) was placed in the wells (instead of the sample) for the positive control and 200 microliters of 3.33% DMSO in a buffer for the negative control. One trial with three replicates was used in the assay. The protocol above was also followed for the same samples at both 10 and 100 µg/ml (27).

The % inhibitory activities of the samples and the positive control (acarbose) are determined based on the average slope of each replicate using the following formula:

$$\%\text{ Inhibitory Activity} = \frac{\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}}{\text{Slope}_{\text{uninhibited}}} \text{ x } 100\%$$

.

Results and Discussion

Diabetes mellitus (DM) is a chronic metabolic

Figure 1. Photographic images of plants used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines.

disorder of carbohydrate, protein, and fat that is caused by insufficient insulin production by the pancreas or insulin resistance. DM is one of the factors that directly affect the economic stability of families due to expensive health care spending for diabetic loved ones. Moreover, DM challenges the health care systems of many countries, like the Philippines. One of the major concerns in the treatment of diabetes is the increasing cost of conventional drug therapy and diagnostic tests. Hence, needy individuals with diabetes may find it challenging to afford the required medications, and the routine medical examination, particularly the national insurance system (Philippine Health Insurance, Inc.) has limited benefit package for diabetes care in the Philippines. However, there was a law in the country advocating and encouraging the utilization of alternative or traditional medicine (TM) that recognizes the use of local remedies for specific human ailments. It makes it accessible and affordable

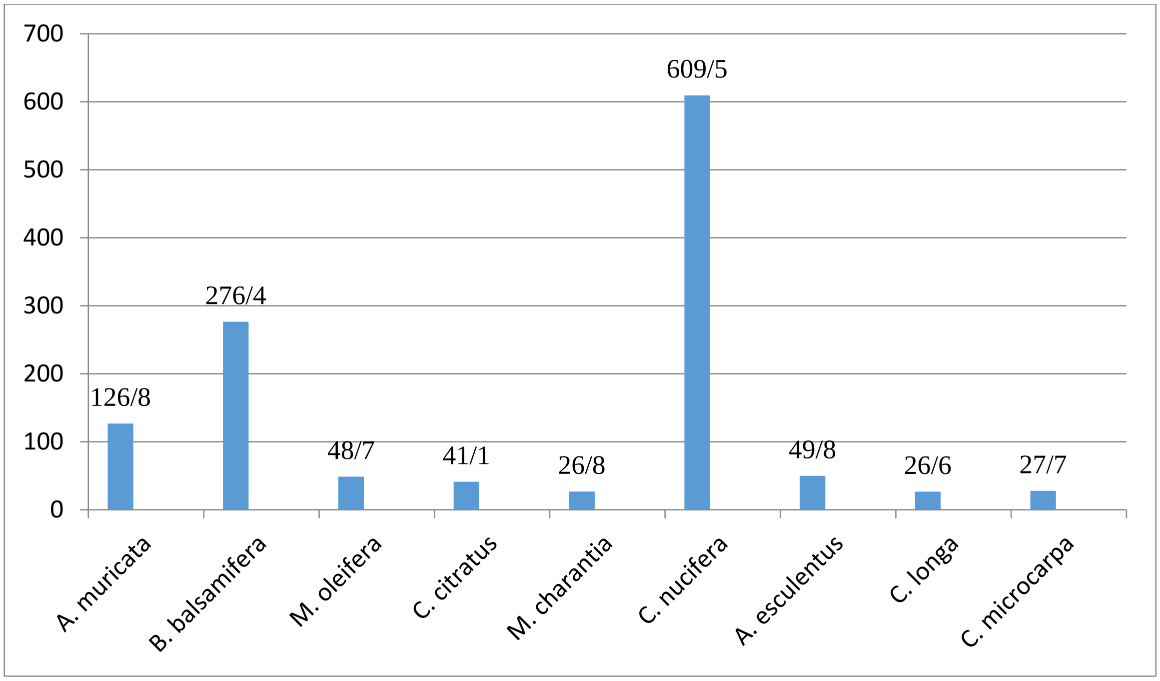

Figure 2. The total phenolic contents (mg GAE/g dw) of plant extracts used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines.

to poor communities but emphasizes the safety and efficacy of its use. Some of the approaches to TM are the recognition of folk knowledge and the use of herbal medicines in the management of certain human conditions such as diabetes.

Similarly, the researchers found eleven (11) well-known traditional healers from rural areas of the Province of Albay, the Philippines, who were interviewed concerning the plants used in the treatment of diabetes. Ten persons of these healers provided the information required for the study. As indicated in Table 1, the informants shared their wisdom concerning the local plants used in the treatment of diabetes, including the mode of preparation and administration. There are about nine plants identified in their local vernacular, which are commonly advised and used by their clients who are suffering from diabetes. With this, the identified plants were further subjected to *in vitro* tests that provided information about their potential antioxidant and hypoglycemic activities.

Plants have been regarded as a potential source of hypoglycemic drugs, and are primarily utilized in several traditional systems of medicine in order to prevent diabetes. Some medicinal herbs used in the treatment of diabetes along with lifestyle management, have been examined for their positive impacts against diabetes. These plants are being further considered because of fewer side-effects and lower cost. Moreover, certain researchers isolated many phytoconstituents that exhibit hypoglycemic effects from antidiabetic plants. Approximately, there are nearly 200 pure compounds taken from herbal sources that have blood glucose lowering capacity. Medicinal herbs capable of controlling blood glucose in diabetes use a particular mechanism that encompasses hindering the absorption of glucose, improving insulin sensitivity, preventing β-cell damage, increasing the insulin release, developing antioxidant defense, attenuating inflammation, modulating carbohydrate metabolism pathway, and regulating insulin-dependent and insulin independent signaling pathways (28). However, the present study identified the common plants used for the treatment of diabetes in the rural areas of the Province of Albay (Figure 1), and initially evaluated their hypoglycemic and antioxidant activities *in vitro*. Nonetheless, further explorations on their potential mechanisms for exhibiting blood glucose control in diabetes will be conducted in the future.

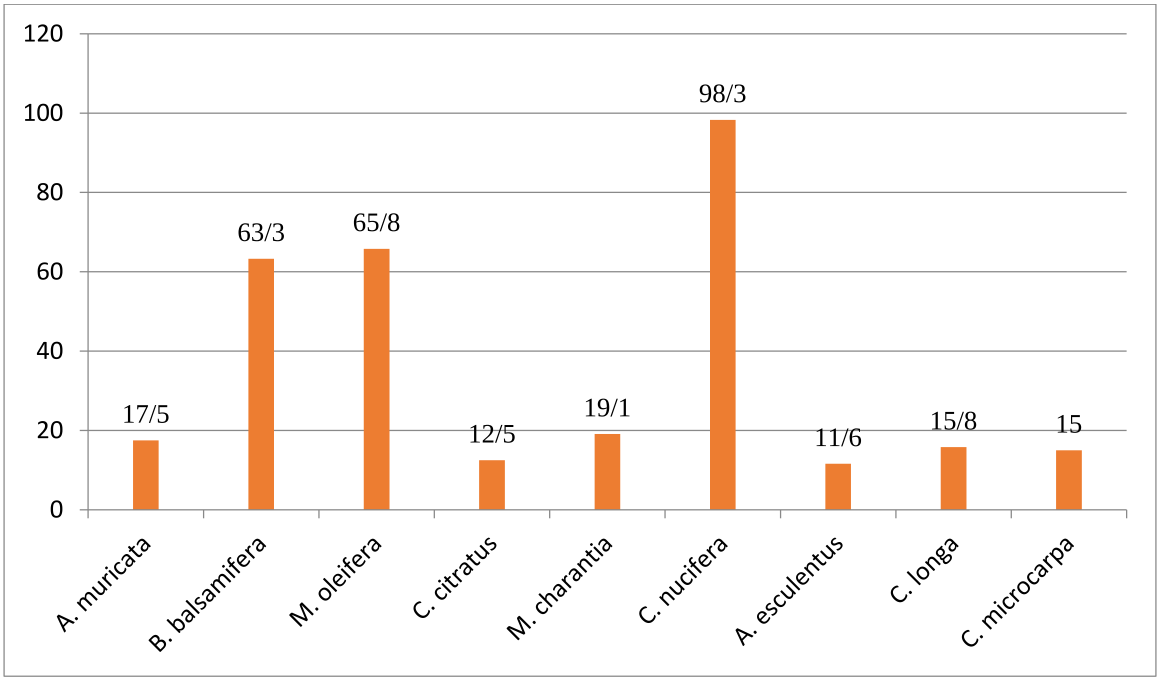

Figure 3. The total flavonoid contents (mg GAE/g dw) of plant extracts used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines.

Total Phenolic Content

Phenolic compounds that are structurally comprised of an aromatic ring, have one or more hydroxyl substituents. They vary from simple phenolic molecules to highly polymerized compounds. However, in spite of the diversity of structure, the compounds are often referred to as “polyphenols’ (29). Phenolic compounds or polyphenols are plant metabolites (30) formed as one of the most abundantly found and widely distributed groups of substances in plants with several known phenol groups (31). The number of total phenolics of nine plants used in the treatment of diabetes by traditional healers was calculated according to a slightly modified version of Folin–Ciocalteu method. As it has been indicated in Figure 2, this number that varied widely in plant materials’ extract ranged from 26.6 to 609.5mg gallic acid equivalents (GAE)/g dry weight (dw). The highest phenolic content was observed in *C. nucifera* L. (609.5mg GAE/gm), while the lowest was found in *C. longa* L. *B. balsamifera* (L.) DC (276.4mg GAE/gm) and *A. muricata* L. (126.8mg GAE/gm) also had very high levels of phenolics. *A. esculentus* (L.) Moench (49.8mg GAE/gm), *M. oleifera Lam* (48.7mg GAE/gm) and *C. citratus* (DC.) Stapf. (41.1mg GAE/gm) had relatively low levels of phenolics, whereas in *C. microcarpa* Bunge (27.7mg GAE/gm), *M. charantia* L. (26.6mg/GAE gm) and *C. longa* L. (26.6mg/GAE gm) phenolics were quite low.

The consumption of high amounts of fruits and vegetables with surprisingly high concentrations of phenolic compounds results in several health benefits (32). Balasundram *et al.* indicated that phenolic compounds could display expansive physiological properties such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects. Furthermore, many researches have indicated the antidiabetic impacts of polyphenols. Polyphenols might affect blood glucose through distinct mechanisms, including the hindrance of glucose absorption in the gut or of its uptake by peripheral tissues (33). In diabetes mellitus (DM), the presence of high blood glucose can inactivate antioxidant enzymes such as SOD, CAT and glutathione peroxidase (GPx) by glycating these proteins. The result will be the production of oxidative stress, which in turn, leads to lipid peroxidation (34). Hence, making phenolics as an antioxidant supplement is significant because it might

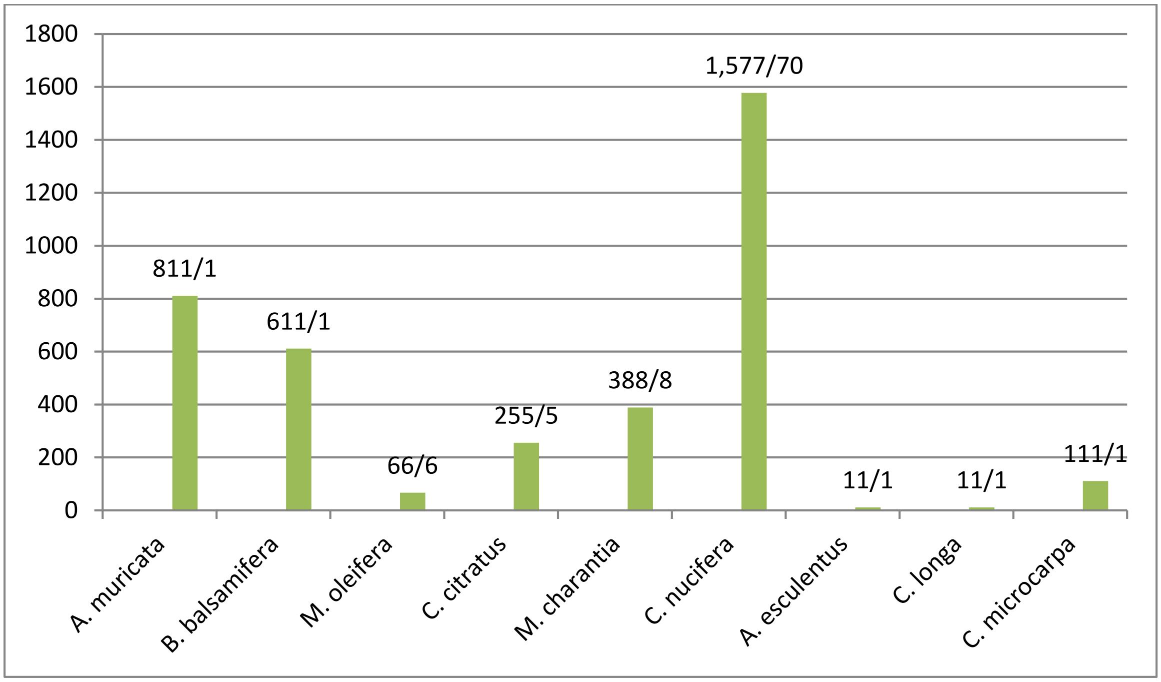

Figure 4. The total tannin contents (mg GAE/g dw) of plant extracts used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines.

contribute to the rise of enzyme activities and attenuation of lipid peroxidation in DM (35). Individually, plants rich in phenolic content possessed greater antioxidant properties than vitamins and synthetic antioxidants (36).

Total Flavonoid Content

One of the major dietary phenolic compounds aside from phenolic acids and tannins are the flavonoids (37). Flavonoids comprise the widest group of plant phenolics, which account for more than half of the eight thousand polyphenols that are naturally found in plants. It has the basic skeleton of diphenylpropanes (C6 + C3 + C6) with distinct oxidations of the central pyran ring (38). Flavonoids are ubiquitously present in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis, and honey (39).

Similarly, flavonoid compounds have been found in the extract of plants used by traditional healers in the treatment of diabetes. As indicated in Figure 3, the number of total flavonoids of nine plants, measured by slightly modified aluminum chloride colorimetric assay, significantly varied in plant materials' extract and ranged from11.6 to 98.3mg gallic acid equivalents (GAE)/g dry weight (dw). The highest level of flavonoids was found in *C. nucifera* L. (98.3mg GAE/gm), while the lowest level was observed in *A. esculentus* (L.) Moench. Relatively high flavonoid contents were also seen in the extract of *M. oleifera Lam* (65.8mg GAE/gm) and *M. balsamifera* (L.) DC (63.3mg GAE/gm), whereas in *M. charantia* L. (19.1mg GAE/gm), *A. muricata* L. (17.5mg GAE/gm), *C. longa* L. (15.8mg GAE/gm), *C. microcarpa* Bunge (15mg GAE/gm), *C. citratus* (DC.) Stapf. (12.5mg GAE/gm) and *A. esculentus* (L.) Moench (11.6mg GAE/gm) flavonoids were quite low. Since flavonoids comprise the largest group of plant phenolics, they are the most prevalent and widely distributed group of phenolic compounds that are found in roughly entire plant parts, particularly the photosynthesizing plant cells (40). Hence, fruits and vegetables are the primary dietary sources of these flavonoids. Consumption of flavonoid-rich foods might be beneficial due to their significant biological properties that improve human health and contribute to the reduction of the risk of ailments. Moreover, dietary flavonoids might assist the human body in guarding against free radicals by supplementing the body's antioxidant defenses (41). Furthermore, flavonoids are considered as 'nutraceutical' (42), a term coined in

Table 1: Ethnobotanical information of the plants used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines, and the frequency of citation of remedy.

Plant species	Vernacular/ local name	Part used	Mode of preparation and administration	Frequency
Annona muricata	Guyabano	Leaves	Boiling[4] of fresh leaves[1] and decoction is taken orally	4
Blumea balsamifera	Lakad bulan	Leaves	Boiling[4] of fresh leaves and decoction is taken orally	1
Moringa oleifera	Kalunggay	Fruits	Boiling[4] of fruits and decoction is taken orally	1
Cymbopogon citratus	Tanglad	Leaves-rhizomes	Boiling[4] of 7-8 pieces of fresh leaves including the rhizomes and decoction is taken orally	1
Momordica charantia	Mariguso	Fruits	The fresh fruits are sliced and eaten[2]	2
Cocos nucifera	Niyog	Roots	Boiling[4] of air-dried roots and decoction is taken orally	1
Abelmoschus esculentus	Okra	Fruits	Soaking[3] of fresh fruits in water and then only water is taken orally	2
Curcuma longa	Dulaw	Rhizomes	Boiling[4] of chopped rhizomes; amount (water and plant material) not specified	1
Citrofortunella microcarpa	Suwa	Fruits	Juicing 3-8 pieces of the fruits and mixed in a glass of warm water and taken orally	1

[1]number of guyabano leaves and amount of water to be added varies accordingly with the suggestions of the four traditional healers (informants): (1) 7 pieces of leaves, amount of water not specified, (2) no specified number of pieces of leaves and amount of water, (3) 9 pieces of leaves in 2 cups of water, allowing 1 cup to evaporate and the remainder is taken, (4) 40 pieces of leaves in 5 cups of water, allowing 4 cups to evaporate and the remainder is taken.
[2]preparation varies accordingly with the suggestions of the traditional healers; (1) thinly sliced and placed in a container with salt and water for a few minutes. Then, water is drained, and fruit is eaten, (2) sliced fruit added with calamansi juice; with no specified amount of the fruit and calamansi.
[3]soaking of okra fruit, amount of water and duration varies accordingly with the suggestions of the two traditional healers: (1) 7 pieces of fruit in a pitcher of water, and then water is consumed within the day, (2) fruit is cut into halves and soaked in water overnight before its consumption.
[4]no specified time duration of boiling.

1979 by Stephen DeFelice and defined “like food or parts of food that provide medical or health benefits, including the prevention and treatment of disease.” With this, extracts of nine plants used by traditional healers in the treatment of diabetes that contain a considerable amount of flavonoids may be further investigated for its potential inclusion as one of the nutraceuticals.

Table 2: Percentage of inhibition activity of nine plants used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines against the DPPH free radicals.

	Inhibition of DPPH (%)									
Concentration (mcg/mL)	*A. muricata*	*B. balsamifera*	*M. oleifera*	*C. citratus*	*M. charantia*	*C. nucifera*	*A. esculentus*	*C. longa*	*C. microcarpa*	Ascorbic acid
5	19.05	31.97	52.93	50.12	24.17	39.72	55.37	38.62	52.93	56.23
10	20.35	33.89	55.63	50.89	34.83	44.01	57.67	35.37	55.63	64.05
20	25.19	54.09	60.68	56.09	35.85	47.22	54.82	38.45	60.68	96.23
50	38.76	63.81	66.49	58.90	39.11	64.92	51.45	44.23	66.49	96.53
100	40.64	90.36	57.72	59.00	38.88	88.57	50.29	40.54	57.73	97.37
IC_{50} value	125.707	28.86	-	-	171.904	23.533	94.184	266.543	34.673	3.591
		0								

Table 3: Percentage of inhibition activity of nine plants used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines against the Alpha-glucosidase.

	Inhibition of α-Glucosidase (%)								
Concentration (μg/mL)	*A. muricata*	*B. balsamifera*	*M. oleifera*	*C. citratus*	*M. charantia*	*C. nucifera*	*A. esculentus*	*C. longa*	*C. microcarpa*
1	-3.64	11.73	-6.78	-4.94	-11.88	13.40	-2.50	-3.29	9.57
10	-10.75	92.44	-17.07	-1.97	-4.043	80.04	2.37	-0.40	-7.41
100	-19.95	98.14	-13.14	-4.29	-14.25	99.20	-10.21	-4.86	-16.01

Total Tannin Content

Vegetable tannins could be categorized as water-soluble phenolic compounds whose molecular weight ranges from 500 and 3000 Daltons that are found in large numbers of plant foods. There are two sorts of tannins, *i.e.*, hydrolyzable and non-hydrolysable or condensed tannins. Hydrolyzable tannins (HT) contain either gallotannins or ellagitannins, and are usually found in minimal levels in plants. Condensed tannins/ proanthocyanidins (PA) are considered as a group of naturally occurring polyphenolic bioflavonoids. They are the polymerized products of flavan-3-ols and flavan-3, 4-diols or a combination of the two (43). Moreover, they are more widespread in the plant kingdom than hydrolyzable tannins (44).

The present investigation found that the extracts of nine plants used in the treatment of diabetes contain tannins. As depicted in Figure 4, the number of total tannins of the nine plants, measured by slightly modified Folin–Ciocalteu method, varied widely in plant materials' extract and ranged from 11.1 to 1,577.70mg GAE/g dry weight (dw). The greatest amount of tannins was observed to be in *C. nucifera* L., while the lowest level was in *A. esculentus* (L.) Moench and *C. longa* L. The extracts of *A. muricata* L. (811.1mg GAE/gm), *B. balsamifera* (L.) DC (611.1mg GAE/gm), *M. charantia* L. (388.8mg GAE/gm), *C. citratus* (DC.) Stapf. (255.5mg GAE/gm) and *C. macrocarpa* Bunge (111.1mg/ GAE/gm) also had high levels of tannins. On the other hand, low levels of tannins were seen in the plant material extracts of *M. oleifera Lam* (66.6mg GAE /gm), *C. longa* L. (11.1mg GAE/gm) and *A. esculentus* (L.) Moench (11.1mg GAE/gm).

Table 4: IC50 values of nine plants used for the treatment of diabetes by traditional healers in the Province of Albay, Philippines against Alpha-glucosidase.

Plants	IC50 values (µg/ml)
A. muricata	-
B. balsamifera	4.97
M. oleifera	-
C. citratus	-
M. charantia	-
C. nucifera	13.36
A. esculentus	-
C. longa	-
C. microcarpa	-

With this, the plants used as hypoglycemic remedies suggested by the traditional healers with relatively high levels of tannins could exhibit certain biological activities. Primarily, food tannins are found to have antioxidant properties. Moreover, they have been considered to be cardioprotective, anti-inflammatory, anti-carcinogenic, and antimutagenic among others. Furthermore, tannins are found to be a potential drug for non-insulin dependent diabetes mellitus (NIDDM), as it shown to enhance glucose uptake. They inhibit adipogenesis and are able to improve the pathological oxidative condition of a diabetic case, and they also exhibit an anti-hyperglycemic activity in streptozotocin-induced diabetic rats (44).

DPPH (2,2-diphenyl1-1-picryl-hydrazyl) Free Radical Scavenging Activity

An antioxidant is a beneficial substance capable of preventing the existence of high levels of free radicals in biological systems (45), whereas a low level of plasma antioxidants might increase the risk of developing human diseases. Free radicals and other reactive oxygen species (ROS) are constantly created through natural physiological processes, especially in pathological conditions (46). Moreover, the human body might generate extreme free radicals because of some modifiable factors such as exposure to stress, smoking, drugs, and diet. If they remain undamaged, they might result in pathological conditions or diseases, including diabetes. However, certain defense mechanisms might react to scavenge free radicals and defend the body against the damaging attack of free radicals. The detoxification of ROS in cells to non-reactive forms is carried out by enzymatic and non-enzymatic systems which constitute the antioxidant defense mechanisms.

However, in the case of progressing diabetes, circulating levels of scavengers are impaired that are potentially caused by oxidative stress leading to life-threatening complications in which antioxidants have been regarded a treatment (46). Hence, the plants used by traditional healers in the treatment of diabetes have also been investigated for their potential free radical scavenging effects or antioxidant activities. Antioxidant could exhibit their effect by unfalteringly reacting with ROS, quenching them, or chelating catalytic metal ions (47).

The scavenging effects of nine plants' extracts on DPPH free radicals were compared with standard anti-oxidant, ascorbic acids. The DPPH scavenging activity test is an appropriate method to assess the antioxidant capacity of fruit and vegetable juices or extracts. It is regarded as easy and accurate (48), and is a stable free radical (49). The radical form of DPPH is absorbed in 517nm, but upon reduction with antioxidant, its absorption diminishes because of the formation of DPPH-H, its non-radical state (49). In this study, hydrogen-donating antioxidants could exhibit free radical scavenging activities which are expressed as a percentage of inhibition. The results have been indicated in Table 2.

This research revealed that the DPPH free radical scavenging activity of nine plants varied from 38.88 to 90.36% at the concentration of 100 µg/mL. The result revealed that the plant extract of *B. balsamifera* (L.) DC had the greatest radical scavenging activity with 90.36% followed by *C. nucifera* L. with 88.57 %, whereas ascorbic acid, as the reference control, exhibited a 97.37% radical scavenging activity at the same concentration. In both *B. balsamifera* (L.) DC and *C. nucifera* L., the radical scavenging activity increased with the rise of the concentration of the extract. Hence, the reduction in the concentration of DPPH is rooted in scavenging activity of the extracts of *B. balsamifera* (L.) DC and *C. nucifera* L. The IC50 value of *B. balsamifera* (L.) DC and *C. nucifera* L. extract were determined as 28.860 and 23.533 µg/ml, respectively. Meanwhile, ascorbic acid was 3.591 μg/ml. Furthermore, some other researchers have indicated the positive relationship between free radical scavenging activity and the total phenolic compound. The radical scavenging activity increased with the rise of the phenolic compound content. Thus, a high correlation between DPPH radical scavenging potential and total phenolic content was evident (50).

Therefore, these results suggest that high radical scavenging activity is presented by the extracts with high total phenolic contents which could be associated with the inherent characteristic of phenolic compounds that contributes to their electron transfer or hydrogen donating capacity (51). Similarly, the extracts of *B. balsamifera* (L.) DC and *C. nucifera* L. were among the nine plants used by the traditional healers in the treatment of diabetes were found to be containing high phenolic contents that enable them to exhibit high radical scavenging activity. However, *B. balsamifera* (L.) DC appeared to be as potent as ascorbic acid with a maximum inhibition of 90.36% at the concentration of 100 μg/L which is comparable to 97.37% for ascorbic acid at the same concentration. Hence, the results also indicates that the use of *B. balsamifera* (L.) DC in the treatment of diabetes might potentially prevent its complications caused by oxidative stress via increasing the circulating scavengers or antioxidants. Furthermore, the increase in free radical scavenging activity of *B. balsamifera* (L.) DC could be explained by the presence of higher phenolic contents and the essential nature of phenolic compounds from its leaf extract. On the other hand, the values of the scavenging activity of the other seven plants under investigation against DPPH appeared to be moderate, either because of low concentrations of antioxidants from their extract or due to the antagonistic behavior of the active compounds that inhibit the antioxidant effects (52).

Alpha-Glucosidase Inhibition Activity

The treatment management goal of patients with DM is to keep the roughly normal levels of blood glucose control in both the fasting and postprandial states. Several studies have been conducted to investigate various natural resources concerning their capacity to hinder the production of glucose from carbohydrates in the gut or glucose absorption from the intestine. The alpha-glucosidase is able to divide the disaccharides into simpler sugars in order to be easily available to be absorbed in the intestines. However, inhibiting its activity, in the human digestive tract is reckoned beneficial for controlling diabetes by curtailing the uptake of glucose which is decomposed from starch by this enzyme (53). Hence, a safe and effective inhibitor of alpha-glucosidase has been explored.

In the present research, the researchers examined the inhibitory activity against alpha-glucosidase of the nine plants identified, which are used by traditional healers in the treatment of diabetes. The plant extracts were investigated for their a-glucosidase inhibitory activities by assessing their IC50 values. The IC50 value is the half maximal inhibitory concentration that resembles the capacity of an inhibitor such as plant extracts required for 50% inhibition of its targeted enzyme (like alpha-glucosidase). It is widely used as a measure to develop such an inhibitory efficacy. Table 3 shows the percentage inhibition of nine plants at 1, 10, and 100 μg/ml concentrations, and Table 4 presents the IC50 values. On the other hand, acarbose was used as the reference drug, which displayed 94% inhibition against alpha-glucosidase at a concentration of 1,000 μg/ml. The results of the present study revealed that only two out of the nine plants could exhibit the inhibitory potency which was displayed by *B. balsamifera* (L.) DC and *C. nucifera* L. with IC50 values of 4.97 and 13.36, respectively. These values are comparable with the IC50 value of acarbose which is 38.25 mg/ml.

The inhibitory effect of these two plants might be associated with the abundant presence of essential phytochemical compounds found in this study. As a good source of antioxidants, flavonoids have been reported to potentially prevent oxidative stress, which adversely impairs the function of pancreatic beta-cell function, thus minimizing the incidence of type 2 diabetes (53). Moreover, recent studies found that phenolic compounds could act on ATP sensitive K+ channels and control blood glucose level (54). Similarly, Kumari & Jain reported that tannins have indicated their capacity to enhance glucose uptake and inhibit adipogenesis. Furthermore, their ability to improve the pathological oxidative state of a diabetic situation has been reported. The results of the present research suggest that *B. balsamifera* (L.) DC and *C. nucifera* L. are potential potent alpha-glucosidase inhibitors. Hence, they are capable of controlling diabetes by curtailing the absorption of glucose which is decomposed from starch by this enzyme. Moreover, a higher intake of flavonoid, phenolic, and tannin-rich foods among diabetic patients might result in the protection against the occurrence of long-term complications of DM. However, the evaluation of the inhibitory activity of these plants found *in vitro* requires further research to establish findings relevant to the human body. Nevertheless, the results endorse the medicinal significance of these plants, and confirm that they are beneficial for maintaining an acceptable glycemic state. Moreover, they are essential in controlling diabetes and minimizing the associated complications.

Conclusion

The present study found that there were nine plants commonly used by traditional healers in the treatment of diabetes in the Province of Albay, Philippines. It also suggests that *B. balsamifera* (L.) DC leaf and *C. nucifera* L. root extracts are among the nine plants that exhibit potent antioxidant activities and might be used as sources of natural antioxidants that would help maintain the physiological status of human health, and prevent and treat various diseases. Furthermore, these two plants possess the inhibitory activity against alpha-glucosidase. Hence, they are capable of controlling the blood glucose level of the human body. Moreover, their extracts contain relatively high bioactive compounds that potentially account for their biological and pharmacological activities.

On the other hand, further exploration is necessary to establish other mechanisms pertaining to the controlling of blood glucose by these plants, particularly for the *B. balsamifera* (L.) DC leaf and *C. nucifera* L. root extracts. Also, the findings warrant other extraction methods and further phytochemical analysis to isolate the essential phytoconstituents which are primarily responsible for the exhibition of these therapeutic activities. In conclusion, the results of the present study scientifically support the efficacy of the use of *B. balsamifera* (L.) DC leaf and *C. nucifera* L. root extracts in the traditional alternative medicine for treating diabetes.

Acknowledgment

The authors are grateful to Philippine Council for Health Research and Development (PCHRD), the Bicol Consortium Health Research and Development (BCHRD), and the Bicol University Research Development and Management Division (BURDMD) for providing them with financial support to conduct the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS medicine. 2006;3(11):e442.
2. Tan GH. Diabetes care in the Philippines. Annals of global health. 2015;81(6):863-9.
3. Prasanna G, Saraswathi N, Devi BP. Diabetes mellitus–An ethnomedicinal perspective. Journal of Pharmacy Research. 2011;4(7):2371-6.
4. World Health Organization. Diabetes action now: an initiative of the World Health Organization and the International Diabetes Federation; 2004.
5. Higuchi M. Costs, availability and affordability of diabetes care in the Philippines. Tokyo, Japan. 2009 Mar.
6. di Sarsina PR, Alivia M, Guadagni P. Widening the paradigm in medicine and health: person-centred medicine as the common ground of traditional, complementary, alternative and non-conventional medicine. InHealthcare Overview. Springer, Dordrecht. 2012; pp. 335-53.
7. Moghadamtousi S, Fadaeinasab M, Nikzad S, Mohan G, Ali H, Kadir H. Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. International journal of molecular sciences. 2015;16(7):15625-58.
8. Vinoth B, Manivasagaperumal R, Balamurugan S. Phytochemical analysis and antibacterial activity of Moringa oleifera Lam. Int J Res Biol Sci. 2012;2(3):98-102.
9. Saraswathy A, Kumar KS, Shakila R, Ariyanathan S. Pharmacognostic Evaluation of Roots of Cocos nucifera linn. Pharmacognosy Journal. 2010;2(12):498-501.
10. Pang Y, Wang D, Fan Z, Chen X, Yu F, Hu X, et al. Blumea balsamifera—A phytochemical and pharmacological review. Molecules. 2014;19(7):9453-77.
11. Kubola J, Siriamornpun S. Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. Food chemistry. 2008;110(4):881-90.
12. Chanchal DK, Alok S, Kumar M, Bijauliya RK, Rashi S, Gupta S. A Brief Review on Abelmoschus esculentus linn. okra. International Journal of Pharmaceutical Sciences and Research. 2018;9(1):58-66.
13. Ekpenyong CE, Akpan EE, Daniel NE. Phytochemical Constituents, Therapeutic Applications and Toxicological Profile of

Cymbopogon citratus Stapf (DC) Leaf Extract. Journal of Pharmacognosy and Phytochemistry. 2014;3(1).
14. Arutselvi R, Balasaravanan T, Ponmurugan P, Saranji NM, Suresh P. Phytochemical screening and comparative study of anti microbial activity of leaves and rhizomes of turmeric varieties. Asian Journal of Plant Science and Research. 2012;2(2):212-9.
15. Ashraf K. A comprehensive review on Curcuma longa Linn.: Phytochemical, pharmacological, and molecular study. International Journal of Green Pharmacy (IJGP). 2018;11(04).
16. Roowi S, Crozier A. Flavonoids in tropical citrus species. Journal of agricultural and food chemistry. 2011;59(22):12217-25.
17. Bhat R, Kamaruddin NS, Min-Tze L, Karim AA. Sonication improves kasturi lime (Citrus microcarpa) juice quality. Ultrasonics sonochemistry. 2011;18(6):1295-300.
18. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). Journal of advanced pharmaceutical technology & research. 2011;2(1):3.
19. Kumar DS, Sharathnath KV, Yogeswaran P, Harani A, Sudhakar K, Sudha P, et al. A medicinal potency of Momordica charantia.
20. Salim AS, Simons AJ, Waruhiu A, Orwa C, Anyango C. Agroforestree database: a tree species reference and selection guide. ICRAF, Nairobi (Kenya); 1998.
21. Tomar A. Medicinal use of Abelmoschus esculentus (Linn.) Moench.(Bhindi) to cure fever. Journal of Pharmacognosy and Phytochemistry. 2017;6(4):596-7.
22. Cheong MW, Chong ZS, Liu SQ, Zhou W, Curran P, Yu B. Characterisation of calamansi (Citrus microcarpa). Part I: Volatiles, aromatic profiles and phenolic acids in the peel. Food chemistry. 2012;134(2):686-95.
23. Hossain MD, Sarwar MS, Dewan SM, Hossain MS, Shahid-Ud-Daula AF, Islam MS. Investigation of total phenolic content and antioxidant activities of Azadirachta indica roots. Avicenna journal of phytomedicine. 2014;4(2):97.
24. Tambe VD, Bhambar RS. Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus tiliaceus Linn. wood extracts. Journal of pharmacognosy and phytochemistry. 2014;2(4):41-7.
25. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002;10(3).
26. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. Journal of Taibah University for Science. 2015 Oct 1;9(4):449-54.
27. Anam K, Widharna RM, Kusrini D. α-glucosidase inhibitor activity of Terminalia species. Int J Pharmacol. 2009;5(4):277-80.
28. Eddouks M, Bidi A, El Bouhali B, Hajji L, Zeggwagh NA. Antidiabetic plants improving insulin sensitivity. Journal of Pharmacy and Pharmacology. 2014;66(9):1197-214.
29. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food chemistry. 2006;99(1):191-203.
30. Song FL, Gan RY, Zhang Y, Xiao Q, Kuang L, Li HB. Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. International Journal of Molecular Sciences. 2010;11(6):2362-72.
31. Harborne JB. General procedures and measurement of total phenolics. Methods in plant biochemistry. 1989;1:1-28.
32. Parr AJ, Bolwell GP. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture. 2000;80(7):985-1012.
33. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity. 2009;2(5):270-8.
34. Kennedy AL, Lyons TJ. Glycation, oxidation, and lipoxidation in the development of diabetic complications. Metabolism. 1997;46:14-21.
35. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of nutritional biochemistry. 2002;13(10):572-84.
36. Zhang Y, Li X, Wang Z. Antioxidant activities of leaf extract of Salvia miltiorrhiza Bunge and related phenolic constituents. Food and Chemical Toxicology. 2010;48(10):2656-62.
37. King AM, Young G. Characteristics and occurrence of phenolic phytochemicals. Journal of the American Dietetic Association. 1999;99(2):213-8.
38. Harborne JB. The flavonoids: advances in research since 1980. Springer; 2013 Nov 11.
39. Cushnie TT, Lamb AJ. Antimicrobial activity of flavonoids. International journal of antimicrobial agents. 2005;26(5):343-56.
40. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal. 2013;2013.
41. Tripoli E, La Guardia M, Giammanco S, Di Majo D, Giammanco M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food chemistry. 2007;104(2):466-79.
42. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. Tropical Journal of Pharmaceutical Research. 2008;7(3):1089-99.
43. Kumari M, Jain S. Tannins: An antinutrient with positive effect to manage diabetes. Research Journal of Recent Sciences ISSN. 2012;2277:2502.
44. Haslam E, Lilley TH, Cai Y, Martin R, Mangnolato D. Traditional herbal medicines-the role of polyphenols. Planta medica. 1989;55(01):1-8.
45. Yahya AH, Chong GH, Tan CP. The phytochemical properties of a new citrus hybrid (Citrus hystrix× Citrus microcarpa). Science Asia. 2014;40:121-4.
46. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, et al. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology. 2003;84(2-3):131-8.
47. Ujwala W, Vijender S, Mohammad A. In vitro antioxidant activity of isolated tannins of alcoholic extract of dried leaves of Phyllanthus amarusschonn and Thonn. Intl. J. Drug Dev. Res. 2012;4(1):274-85.
48. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food chemistry. 2006;94(4):550-7.
49. Harini R, Sindhu S, Sagadevan E, Arumugam P. Characterization of in vitro antioxidant potential of Azadirachta indica and Abutilon indicum by different assay methods. J Pharm Res. 2012;5:3227-31.
50. Ravichandran K, Ahmed AR, Knorr D, Smetanska I. The effect of different processing methods on phenolic acid content and antioxidant activity of red beet. Food Research International. 2012;48(1):16-20.
51. Nguyen QV, Eun JB. Antioxidant activity of solvent extracts from Vietnamese medicinal plants. Journal of Medicinal Plants Research. 2011;5(13):2798-811.
52. Surveswaran S, Cai YZ, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food chemistry. 2007;102(3):938-53.
53. Bhandari MR, Jong-Anurakkun N, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata, Haw.). Food Chemistry. 2008;106(1):247-52.
54. Gandhi GR, Ignacimuthu S, Paulraj MG. Solanum torvum Swartz. fruit containing phenolic compounds shows antidiabetic

and antioxidant effects in streptozotocin induced diabetic rats. Food and Chemical Toxicology. 2011;49(11):2725-33.