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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIDIABETIC EVALUATION OF LEAF EXTRACTS FROM *DIOSPYROS BLANCOI* A. DC.

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ABSTRACT: The objective of the study was to determine the phytochemicals present in the leaf extracts of *Diospyros blancoi* A. DC and to evaluate its antioxidant and antidiabetic activities. Qualitative phytochemical tests were used to detect the presence of bioactive compounds present in leaf extracts of *D. blancoi*. Antioxidant activity was measured through diphenyl-1-picrylhydrazyl assay, and antidiabetic potential was done using alloxan induced white mice. Based on the results, the following bioactive constituents are present in the leaves: alkaloids, flavonoids, tannins, terpenoids, saponins, anthraquinones, steroids, and glycosides in both ethanol and water extracts of *D. blancoi*. Moreover, results showed that the ethanolic extract had better antioxidant activity compared to water extract. Furthermore, results also showed that both ethanolic and waterleaf extracts had significant antidiabetic effects to alloxan-induced diabetic white mice as shown in their ability to reduce blood glucose level. Results indicate that extracts of *D. blancoi* leaves are potential sources of natural antioxidant and antidiabetic compounds and could have potential use in the management of diabetes.

INTRODUCTION: Traditional and folklore medicine play an important role in health services around the globe. The significance of plants in medicine remains even of greater importance with the current globalization to obtain drugs from plant sources due to high safety, efficacy, and economy [1]. The search for new plant-derived drugs has been receiving renewed interest among researchers throughout the world given discovering new drugs. Studies on plant remedies not only serve to make the public more conscious regarding sources of alternative medicines but also to open more avenues for future drug discovery [2].

In this growing interest, many of the phytochemical bioactive compounds from medicinal plants have been studied intensively and have shown many pharmacological activities [3]. Screening of various bioactive compounds from plants has led to the discovery of new therapeutic drugs which have adequate protection and treatment roles against multiple diseases. Micro-scale bioassay techniques provide a frontline screen and are attractive because of simplicity, rapidity, cost-effectiveness and reasonable reliability, therefore are used extensively for the screening of biological activity of plant materials [4].

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Tropical countries like the Philippines are abundant in medicinal plants. Herbal knowledge from the local indigenous community has long been the basis for investigating the potential of plants as therapeutic agents [5]. *Diospyros blancoi* is not commonly known as alternative medicine in most areas in the Philippines but is commonly used in Claver, Surigao Del Norte to cure some illnesses. A decoction of leaves of *D. blancoi* can cure dysentery and fever, diarrhea, and has antibacterial properties. Moreover, *D. blancoi* is used to treat chest colds, scars, stress, hypertension, heart ailments, and diabetes.

The objective of this study was to determine the secondary metabolites using a standard protocol for qualitative phytochemical screening and to determine the antioxidant and antidiabetic activities of *D. blancoi* to validate the claims of local healers from Claver, Surigao Del Norte Philippines.

MATERIALS AND METHODS:

Identification and Collection of Plant Samples: Fresh leaves of *D. blancoi* were collected from Claver, Surigao del Norte, Philippines (9'29"21 N and 125'50"40 E). Field guide book and taxonomic keys following the list of medicinal plants of Philippines were used to identify the plants used in the area [6-7]. Moreover, complete plant materials of *D. blancoi* were photographed in their natural habitat for proper documentation.

Extract Preparation: Leaf samples (300 g) of *D. balncoi* were thoroughly washed under running tap water to remove unwanted material, rinsed with distilled water, cut into smaller pieces, and boiled in sufficient amount of distilled water for 5 min in low heat. The resulting decoction mixture was filtered, cooled, freeze-dried, and stored in air-tight containers. One kg of fresh plant materials was air-dried for 2-4 weeks, homogenized to a fine powder using an electric blender, and was soaked in 1 lit of 95% ethanol for 72 h, filtered, and concentrated *in-vacuo* to give the ethanolic extract. The hydro-ethanolic extract was also prepared by mixing 100 ml distilled water and 100 ml pure 95% ethanol or 1:1 ratio. The resulting mixture was filtered, concentrated *in-vacuo* using a rotary evaporator, freeze-dried, and weighed to provide the hydro-ethanolic extract. The prepared extracts were used for the phytochemical tests.

Qualitative Analysis for Phytochemical Components: Phytochemical analysis of the leaf extracts of *D. blancoi* was done using standard qualitative methods [8-9]. The compounds analyzed for phytochemicals were alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, and anthraquinones.

DPPH Radical Scavenging Activity: The antioxidant activity of the extracts was measured by the scavenging activity of the stable 2, 2-diphenyl- 1-picrylhydrazyl assay (DPPH) [10]. Free radical scavenging capacity was evaluated on the basis of the scavenging activity of DPPH by measuring the reduction of absorbance at 517 nm. The DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (50, 100 & 500 µg/ml). A corresponding blank sample was prepared, and L-Ascorbic acid (1.0-100 µg/ml) was used as reference standard. The mixture of 1ml methanol and 1 ml DPPH solution was used as a control. The reaction was carried out in triplicate, and the decrease in absorbance was measured at 517nm after 30 minutes in the dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

% Free Radical Inhibition = [(A control – A sample) / A control] × 100

Where A control and A sample are the absorbance values of the control and test sample, respectively. The effective concentration of sample required to scavenge DPPH radical by 50% (EC_{50}) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentration.

Experimental Animals: A total of 24 healthy adult mice of both sexes (20-30 g) were obtained from the rodent laboratory of Caraga State University, Ampayon, Butuan City and were acclimatized for five days to 25 ºC, and 12 h dark/light cycle with free access to commercial rodent food (pellets) and water *ad libitum*. Ethics clearance approval number is (National Ethics Committee) NEC Code: 2018-023-Demetillo.

Induction of Diabetes in Experimental Animal: Alloxan monohydrate (Sigma-Aldrich Chemical Corp. Germany) was dissolved in sterile distilled

water and was then administered by a single intraperitoneal dose (120 mg/kg) body weight) into 12 h fasted mice. The blood samples were taken after 2 days of alloxan injection. Mice screened for diabetes having glycosuria and hypoglycemia with a blood glucose level of above 240 mg/dL were taken for the study. All animals were allowed free access to water and pellet diet. Extracts were administered by oral gavage daily for 21 days from the day of induction. All procedures in the experiments were guided by the observance of animal ethics guidelines from Bureau of Animal Industry.

Experimental Design: All diabetic surviving mice were taken and divided into groups of three mice each: normal control mice (NCR); diabetic control mice (DCR); diabetic mice given ethanol, decoction and hydro-ethanol extracts of (100 mg/kg body weight); diabetic mice given ethanol, decoction and hydro-ethanol extracts of (50 mg/kg body weight); and diabetic mice given standard drug glibenclamide (100 and 50 mg/kg body weight).

Collection of Blood Samples and Blood Glucose Determination: Blood samples were drawn from the tail tip of mice at weekly intervals until the end of the study. This was done by sterilizing the tail with 10% alcohol and then nipping the tail. Blood was then drawn from the tip using blood glucose self-test (Almedicus Co. Ltd., South Korea). Blood glucose determination was done on days 0, 7, 14, and 21 of the study.

All procedures in the experiments were guided by the observance of animal ethics guidelines from Bureau of Animal Industry.

RESULTS AND DISCUSSION: Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, and steroids in all extracts **Table 1**. All the phytochemicals tested were present except anthraquinones which were absent in the water extract. In terms of solvent, ethanolic extracts have abundant phytochemicals compared to water extracts.

TABLE 1: PHYTOCHEMICAL SCREENING OF THE DIFFERENT EXTRACTS OF *D. BLANCOI* LEAVES

Plant extracts	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Cyanogenic glycoside	Anthraquinones
DbD	+	+	++	+	+	+	-
DbHE	+	+	+	+	+	+	-
DbE	+	++	++	++	++	++	+

Key: (+)-present; (++)-abundant; (-)-absent; (DbD, DbHE, DbE)-*Diospyros blancoi* decoction, hydroethanolic and ethanolic extracts respectively.

Phytochemical screening of the leaf extracts of *D. blancoi* revealed the presence of alkaloids, saponins, steroids, tannins, anthraquinones, and flavonoids which are compounds known to have curative activity against several pathogens and therefore could support the traditional use for the treatment of various illnesses in the community. These phytochemicals also exhibited a wide range of activity such as anti-inflammatory, antiviral, antibacterial, antiulcer, anti-allergic, and anti-hepatic action [12]. Alkaloids are basic nitrogenous compounds which are pharmacologically-active and may exhibit tranquilizing and stimulating activities on the nervous system, hypertensive and hypotensive action, vasoconstrictor and vasodilator effects on the cardiac system [13]. Steroids have a chemical structure similar to cholesterols which have been reported to decrease cholesterol absorption and plasma Low-Density Lipoprotein (LDL) values. Saponins which are abundant have been shown to have enormous significance as anti hyper-cholesterol, hypotensive, and have cardiac depressant properties [14]. Furthermore, the existence of different secondary metabolites, especially saponins, tannins, and flavonoids in plants has also been linked to the antimicrobial activities of the plants [15-16]. The result of DPPH free radical scavenging activity on the leaf extracts of *D. blancoi* is shown in **Table 2.** The highest radical scavenging activity was shown by ethanol extract (94.27%). The results obtained were comparable to the standard used (L-ascorbic acid) with a percent inhibition of 96.99. Results revealed that the decoction leaf extract of *D blancoi* obtained the highest EC_{50} value of 63.17 µg/ml radical scavenging (antioxidant) activity, followed by ethanolic extract with an EC_{50} value of 105.38 µg/ml. These results suggest that the plant extract contains components with radical scavenging potential.

TABLE 2: DPPH FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF *D. BLANCOI*

Extracts	500 µg/mL		100 µg/mL		50 µg/mL		EC_{50} µg/mL
	Ave. ABS*	% Antiradical activity	Ave. ABS*	% Antiradical activity	Ave. ABS*	% Antiradical activity	
DbD	0.056	93.99	0.276	71.03	0.622	33.47	63.17
DbE	0.053	94.27	0.315	66.73	0.673	28.11	105.38
DbHE	0.28	69.95	0.571	38.84	0.724	22.74	214.14
AA	0.028	96.99	0.086	90.81	0.464	50.26	21.31

Key: (DbD, DbHE, DbE)-*Diospyros blancoi* decoction, hydroethanolic and ethanolic extracts respectively; AA- Ascorbic acid; * mean of 3 determinations

TABLE 3: MEAN BLOOD GLUCOSE LEVEL OF EACH TREATMENT OF ALLOXAN- INDUCED WHITE MICE

Plant Extracts/ Control	Blood Glucose Level (mg/dL)							
	Initial (0 day)		7^{th} day		14^{th} day		21^{th} day	
	Dose		Dose		Dose		Dose	
	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml
DbD	252	252.4	199.6a	178.9b	119a	110.6b	91.5a	63.3b
DbE	252.5	248.9	146.8a	188.6b	99.8a	126.4b	90.5a	113b
DbHE	260.8	257.4	200.5a	216.2b	144.3a	169.4b	125.1a	145.7b
Glibenclamide	261.5	251.8	149.5a	123.9b	112.5a	98.6b	90.3a	91.4b
DCM	252		247.3ns		249.1ns		247.8ns	
NCM	132.5		137.7ns		134.2ns		137.4ns	

Key: (DCM)- Diabetic control mice; (NCM)- nondiabetic control mice; (a) $P<0.05$ significant decrease as compared to Zero h in a row @ 100mg/ml dose; (b) $P<0.05$ significant decrease as compared to Zero hr in a row @50mg/ml dose; (ns)-not significant

A dose-dependent reduction in Blood Glucose Level (BGL) was observed in alloxan-induced diabetic mice treated with ethanolic, hydroethanolic, and decoction extracts of *D. blancoi*. Following the treatment, the extracts produced a significant reduction ($p<0.05$) in blood glucose levels of the treated diabetic mice. The effect was significantly compared to the standard drug, glibenclamide **Table 3**. A remarkable decrease in blood glucose levels was observed with a dose of 100 mg/kg compared to 50 mg/kg in all plant extracts. Among the extracts tested, it was found that ethanolic showed a gradual decrease in glucose level throughout 21 days, even though the onset of antidiabetic effect with these extracts was seen within 7 days of treatment.

Many studies have shown that many polyphenols contribute significantly to the antioxidant activity and act as highly effective free radical scavengers which are mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals [16]. Flavonoids and tannins are major groups of phenolic compounds that act as primary antioxidants or free radical scavengers. The medicinal effects of plants are often attributed to the antioxidant activity of phytochemical constituents [17]. Many studies confirmed that phenolic constituents, such as flavonoids, phenolic acids, and tannins are well known for their high antioxidant activities and are dominant chain-breaking antioxidants of which most of these are present in the leaves. Moreover, anthraquinones have also shown antioxidant properties. The saponins from plant extracts have already been reported as antibacterial and have potent antioxidant activity [18-19]. Most of the polyphenols contribute significantly to the antioxidant activity and act as highly effective free radical scavengers which are mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals. Another bioactive compound that is abundantly found from the leaf extract of *D. blancoi* is the flavonoids that have been found to possess antioxidant properties [20].

The hypoglycaemic activity observed with ethanolic and water extracts of *D. blancoi* can be accounted to the presence of different phytochemicals which could act synergistically or independently in lowering the blood sugar level. Phytochemical studies have been associated with the hypoglycaemic activity. *Nauclea latifolia*, a plant screened to contain alkaloids, showed a significant decrease of blood glucose levels in alloxan-induced diabetic rats. Flavonoids are known to regenerate the damaged beta cells in the alloxan-induced diabetic rats [21-22]. The reduction in blood glucose of diabetic mice could occur partly by stimulating insulin production from the

pancreatic islets, or it could stimulate insulin production and glucose utilization similar to glibenclamide due to the presence of certain hypoglycemic bioactive components in their ethanolic extracts.

In the present study, the phytochemical analysis of ethanol extract of *D. blancoi* leaf pointed out the presence of above said active principles. This extract could have utilized one of the above mechanisms in exerting its antidiabetic effect. This condition was alleviated by the treatment of the diabetic mice with extracts of *D. blancoi* as the treated mice were healthy and agile at the end of the study. The results of the present study showed the significant hypoglycemic potential of the ethanolic extracts of *D. blancoi* as they reversed the fasting blood sugar of diabetic mice to near normalcy.

CONCLUSION: The results of this chemical screening revealed that *D. blancoi* is rich in secondary metabolites which are potential sources for antioxidant compounds due to their high radical scavenging activity and have antidiabetic properties as shown in their ability to reduce blood glucose level of alloxan-induced diabetic mice. The results indicate the importance of *D. blancoi* as traditional medicine and serve as a basis for further research studies.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest regarding the publication of this paper.

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