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ORIGINAL ARTICLE **OPEN ACCESS**

Screening of the Antioxidant Properties of the Leaf Extracts of Philippine Medicinal Plants Ficus *nota* (Blanco) Merr., *Metroxylon sagu* Rottb., *Mussaenda philippica* A. Rich., *Inocarpus fagifer*, and *Cinnamomum mercadoi* Vidal

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ABSTRACT
In our continued searched for natural antioxidants from Philippine medicinal plants, decoction and ethanol extracts of the leaves of Ficus nota (Blanco) Merr., Metroxylon sagu Rottb., Mussaenda philippica A. Rich, Inocarpus fagifer, and Cinnamomum mercadoi Vidal were investigated for their antioxidant potentials. The C. mercadoi ethanolic (CmE) extract demonstrated the highest amount of total phenolics (570.58 mg GAE) and it correlates well to its strong radical scavenging activity (91.97% at 500 ppm) against 1,1Diphenyl-2-picrylhydrazyl (DPPH) and high antioxidant capacities (149.91 AAE, 209.98 BHTE). The M. sagu decoction (MsD), (403.00 mg GAE, 96.39 AAE, 139.61 BHTE) and ethanolic (MsE), (310.58 mg GAE/g, 73.49 AAE, 102.36 BHTE) extracts are the second and third that showed high amount of phenolics, strong free radical scavenging against DPPH and high antioxidant capacities, respectively. These are followed by decoction extracts of F. nota (FnD) (88.19% at 500 ppm against DPPH, 216.64 mg GAE, 55.52 AAE, 77.05 BHTE), I. fagifer (IfD) (83.64% at 500 ppm against DPPH, 194.48 mg GAE, 63.13 AAE, 88.89 BHTE), C. mercadoi (CmD) (83.64% at 500 ppm against DPPH, 166.03 mg GAE, 80.88 AAE, 88.31 BHTE), and ethanolic extract of I. fagifer (IfE) (89.76% at 500 ppm against DPPH, 131.48 mg GAE, 59.48 AAE, 82.63 BHTE). These findings may support their traditional/ethno-medicinal claims. This study further indicates that the extracts from C. mercadoi, M. sagu, F. nota, and I. fagifer can be used as important sources of natural antioxidants that may offer protection from the harmful effects caused by overproduction of radicals in the body.
Keywords: *medicinal plants, 1,1Diphenyl-2-picrylhydrazyl, total phenolics content, total antioxidant capacity, antioxidants, oxidative stress, traditional medicine.*

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INTRODUCTION

Plants represent a vast source of natural antioxidants. Natural antioxidants are known to control the oxidative stress caused by sunbeams, reactive oxygen species (ROS) and free radicals (FR)[1]. Numerous studies suggests that ROS and FR are involved in high number of diseases such as cancer, accelerated ageing and cardiovascular diseases [1-4]. Plants' natural free radical scavenging molecules such as polyphenols, flavonoids, gluthathione, vitamin E (α-tocopherol), vitamin C, and other endogenous metabolites are known to function as singlet and triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors and synergists which can be used for prevention and treatment of such diseases [5].

In the Philippines, numerous medicinal plants are being used ever since as topical ointments, liniments, poultices for wounds, skin diseases, muscle pains, and aromatherapies, among others [6]. However, to date, only ten Philippine medicinal plants are scientifically approved and recommended for use by the government's Department of Health (DOH), through the Philippine Institute of Traditional and Alternative Health Care (PITAHC), and only five have commercial applications. Some Philippine medicinal plants are formulated and marketed only in a form of herbal and food supplements. For this reason, continued laboratory investigations have been employed on Philippine medicinal plants for their active constituents and antioxidant potentials. Consequently, it is very important to establish a scientific basis

for the traditional/ethno-medicinal claims of these plants as this may offer more prospects in the discovery and development of new drugs from these plants.

Ficus nota (Blanco) Merr., a member of family Moracea., is a small tree endemic to the Philippines and locally known as "tibig". The fruit can be eaten raw when ripe and the young leaves are cooked as vegetables. The water extracted from standing tree is drunk three times daily for fever and applied to relieve muscle pain. The decoction of roots and bark and the water from cut branches are used for urinary tract infection, hypertension and diabetes [7].

Metroxylon sagu Rottb. is a medium height sago palm which contains 80% starch and has long been a staple food for different tribes in South-East Asia [8] and the Manobo tribe in the Province of Agusan del Sur, Philippines [9]. *M. sagu* is used as food during fevers and convalescence. It is used as an excipient for making poultices for shingles while the stem sap is applied to forehead to ease headaches. Its starch is mixed with water for diarrhea and stomach pains while starch paste is applied to burns. The leaf is used to cover fresh or infected sores until they heal. It is mixed together with other herbs in a polyherbal formulation to treat menorrhea and polymenorrhea [8].

Mussaenda philippica A. Rich belonging to the family Rubiaceae, is a shrub that is more or less hairy or nearly smooth [10]. Decoction of its roots and leaves are used for affections of chest and lung while the white, full-grown sepals are used in jaundice and the bark is for stomach ache 9 [11].

Inocarpus fagifer is known as Tahitian chestnut, is a medium size, evergreen tropical tree found in secondary forest and most common along riverbanks, swamps and marshes, and within coastal shorelines [12]. The bark is grated and mixed with coconut milk or bark sap to treat urinary infections in the Solomon Islands and the juice from the mesocarp of green fruits is used in Tonga to treat insect bites and burns. In Fiji, all parts of the tree (root, stem, bark, and leaves) are thought to have various medicinal properties such as to relieve labor pains, pains in the bones, used to treat scabies, stomachache, fish poisoning and also used to stop internal bleeding. The juice squeezed from the fresh leaves is mixed with water and drunk daily to bring down high malarial fever [13].

Cinnamomum mercadoi Vidal, is a small tree locally known as "kalingag" and endemic to the Philippines. Decoction or infusion of the bark used for loss of appetite, bloating, vomiting, flatulence, toothache, headaches, rheumatism, dysentery, to help expel flatus and to facilitate menses; colds, fevers, sinus infections, bronchitis, stomach troubles and tuberculosis. Decoction of leaves also used for expelling gas, used for diarrhea, menorrhagia, dysmenorrhea and neuralgic pains, yeast infections, diabetes, indigestion and treatment of scabies and lice [14].

MATERIALS AND METHODS

Plant materials

Fresh and healthy leaf samples of the medicinal plants were collected from the different localities in the Province of Agusan Del Norte, Philippines. Identification and authentication of the collected test plants was done at the Dept. of Biology and Dept. of Forestry, Caraga State University, Ampayon, Butuan City, Philippines. The fresh plant materials were washed thoroughly under running water, air-dried, homogenized to powdered form, and stored in airtight containers until use.

Plant extraction

The fresh and healthy plant leaf samples was thoroughly washed under running tap water to removed unwanted material, rinsed with distilled water, cut into smaller pieces, and boiled in sufficient amount of distilled water (1:2) for 5 minutes. The resulting decoction mixture was filtered, cooled, freeze-dried, and stored in air-tight containers. The homogenized powdered plant materials were soaked in an adequate amount of 95% ethanol for 72 hours. The resulting mixture was filtered, concentrated *in vacuo* using rotary evaporator, and weighed to provide the ethanol extract.

Spectrophotometric measurements

All the assay ultraviolet absorbance measurements were done using a Lasany Double Beam LI-2800 Microprocessor UV-Vis Spectrophotometer.

DPPH radical scavenging test

The DPPH radical scavenging activity of the test samples were evaluated based on the decrease absorbance of ethanolic DPPH solution at 517 nm [15]. Different concentrations (10, 50, 100, 500, and 1000 μg/mL) of each of the test samples were prepared. The assay mixture contained in a total volume of 1 ml consists of 500 μL of the test sample, 125 μL of freshly prepared DPPH solution and 375 μL of solvent (ethanol). The mixture were mixed vigorously in a vortex mixer for 10 s and incubated at room temperature in the dark (wrapped with aluminum foil) for 30 min. The absorbance was read at 517 nm. In each experiment, the tested sample alone in ethanol was used as blank while the DPPH solution alone in ethanol was used as control. All experiment was carried out in triplicate. L-ascorbic acid was used as a standard. The antioxidant activity (AA) was expressed as:

$$AA\% = [(A_{control} - A_{sample})/A_{control}]x\ 100$$

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 % AA and concentration.

Total antioxidant capacity by phosphomolybdenum method

The total antioxidant activity of the test samples were evaluated by phosphomlybdenum method [16]. This test is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the subsequent formation of a green phosphate/Mo(V) complex at acid pH. A 0.3 ml test sample solution (250 µg/mL), dispensed into screw-capped test tubes was added separately with 3.0 mL reagent solution (0.6M H_2SO_4, 28 mM sodium phosphate, 4mM ammonium molybdate). The screw-capped test tubes were incubated at 95^0 C for 90 min, cooled to room temperature, and the absorbance was measured at 695 nm using a spectrophotometer. Ascorbic acid and butylated hydroxytoluene were used as reference standards and the antioxidant activity was expressed as ascorbic acid equivalents (AAE) and butylated hydroxytoluene equivalents (BHTE) determined from established linear equations. A higher standard equivalents value indicates a higher antioxidant activity. The test was carried out in three trials for each test sample.

Total phenolics content test

The total phenolic contents of the test samples was determined spectrophotometrically using the Folin-Ciocateu method [17]. A 0.1 mL (0.5 mg/mL) of test sample was combined with 2.8 mL of 10% Na_2CO_3 and 0.1 mL of 2N Folin-Ciocalteu reagent. After 40 min. absorbance at 725 nm was measured. Total phenolics was expressed as gallic acid equivalence (GAE) by computing with standard calibration curve constructed for different concentrations of gallic acid (50-400 mg/g).

RESULTS

DPPH radical scavenging assay

The free radical scavenging activity of the leaf extracts was evaluated and expressed in terms of the concentration of the extracts required to reduce by 50% the initial amount of DPPH. The results of this assay are summarized in Table 1 and Figure 1.

Total antioxidant capacity by Phosphomolydenum method

In this assay, crude plant extracts were used in redox-linked reaction whereby the antioxidant present in the plant samples act as the oxidants. The reduction of Mo(VI) to Mo(V) by the antioxidant compound forms an deep green color which can be measured at a wavelength of 695 nm. The trend for molybdenum ion-reducing activities of different plant extracts in the present study is shown in Figures 2 and 3.

Total phenolics content

The total phenolics content of the selected plant extracts was calculated through a linear gallic acid standard curve (y = 0.0011x – 0.0386; R2 = 0.9997) and expressed as Gallic Acid Equivalence (GAE) corresponding to milligram of gallic acid per gram of extract. Figure 4 shows the total phenolics content of the various leaf extracts.

Table 1: DPPH radical-scavenging activities of the plant extracts at various concentrations

Plant/ Standard	*Extracts*	*Code*	*Antiradical activity, %**				*EC_{50}*
			10 ppm	*50 ppm*	*100 ppm*	*500 ppm*	
F. nota	Decoction	FnD	2.43	19.02	42.61	88.19	109.50
	Ethanol	FnE	2.55	6.17	7.74	9.65	>500
M. sagu	Decoction	MsD	26.69	86.57	93.28	92.42	26.97
	Ethanol	MsE	20.04	55.22	86.18	86.38	48.11
C. mercadoi	Decoction	CmD	6.97	18.75	33.78	83.87	154.57
	Ethanol	CmE	44.31	82.37	91.25	91.97	18.87
I. fagifer	Decoction	IfD	6.41	27.85	47.51	83.64	189.57
	Ethanol	IfE	5.90	15.91	29.55	89.76	254.04
M. philippica	Decoction	MpD	0.23	4.45	11.02	58.35	429.54
	Ethanol	MpE	83.34	82.76	83.34	84.35	<10
Ascorbic acid**		AA	25.43	80.33	93.16	98.57	31.06

* - mean of triplicate analysis
** - standard

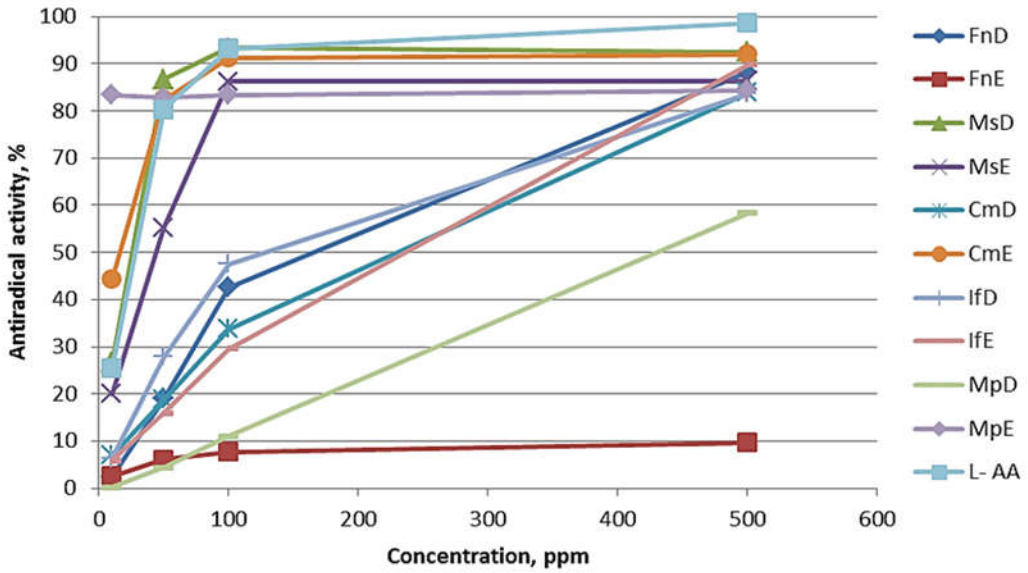

Figure 1: Percent antiradical activity of the plant extracts at various concentrations as compared to Ascorbic acid

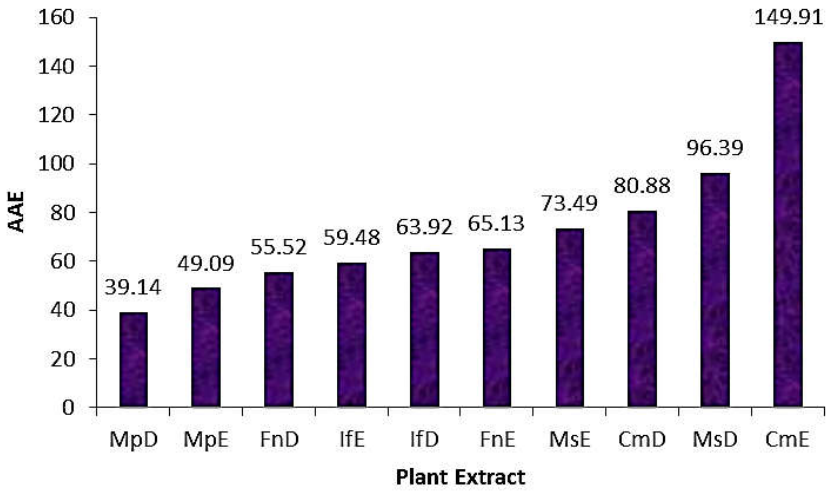

Figure 2: Leaf extracts total antioxidant capacities at 500-ppm concentration expressed as ascorbic acid equivalents (AAE)

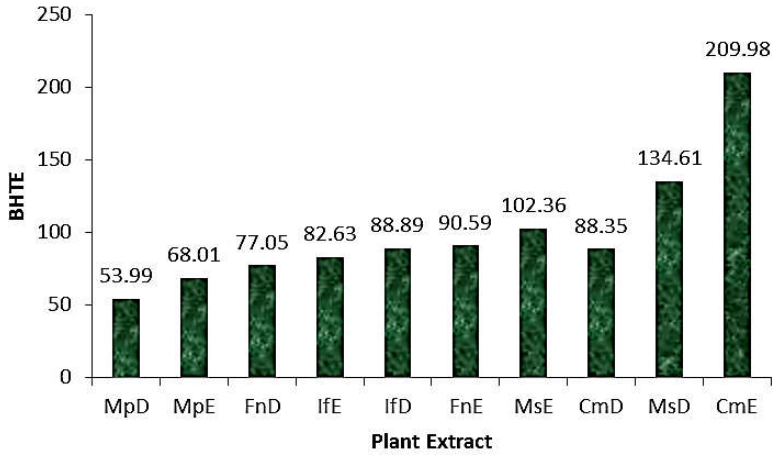

Figure 3: Leaf extracts total antioxidant capacities at 500-ppm concentration expressed as butylated hydroxytoluene equivalents (BHTE)

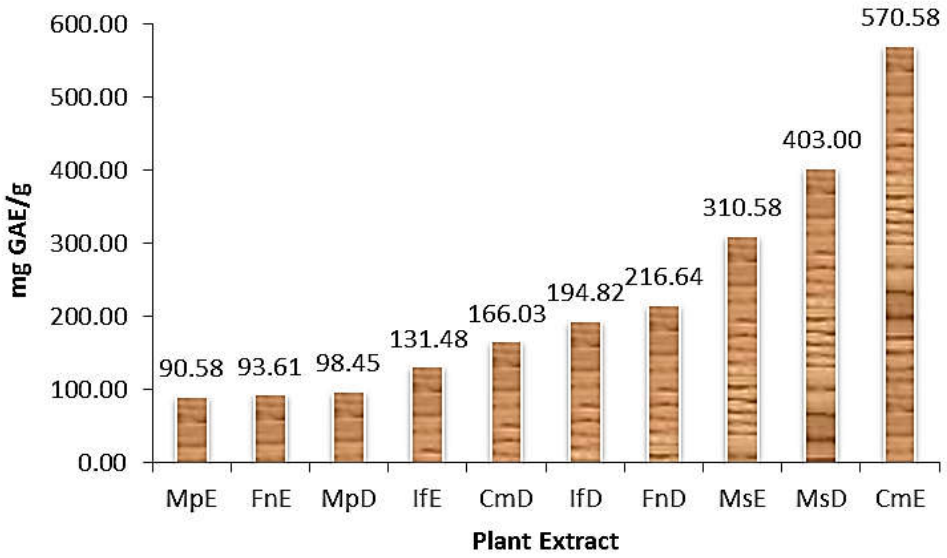

Figure 4: Leaf extracts total phenolic content at 500-ppm concentration expressed as gallic acid equivalence (GAE)

DISCUSSION

DPPH radical scavenging assay

The DPPH is a stable free radical and produces a purple color in ethanol solution and it will be reduced if the antioxidant molecules are present in the leaf extracts, giving rise to the discoloration of the ethanol solution. Thus, a lower EC_{50} value indicates higher DPPH free radical scavenging activity. DPPH was generally used to evaluate antioxidant activity because it is simple, rapid, sensitive, and reproducible procedure [18]. Based on the results obtained from the data in Table 1 and Figure 1, it was observed that most of the plant extracts showed a concentration-dependent DPPH radical scavenging activity, however, with varying degrees of scavenging capacities. As shown by the EC_{50} values, the leaf decoction (MsD) and ethanolic extracts of *M. philippica* (MpE), and *C. mercadoi* (CmE) have greater DPPH radical scavenging activities as compared to the Ascorbic acid (AA) standard. Meanwhile, the ethanolic extract of *M. sagu* (MsE) has almost the same antiradical activity as the standard. Except for the ethanol extract of *F. nota* (FnE), all the other plant extracts also exhibited considerable radical- scavenging activities. Moreover, it is worthy to note that the antiradical activity of the decoction of *I. fagifer* (IfD), *F. nota* (FnD), *C. mercadoi* (CmD) and *M. philipica* (MpD), as well as the ethanolic extract of *I. fagifer* (IfE) have a linear correlation with the given concentrations. These results suggest that these plant extracts contains components with radical scavenging potential.

Total antioxidant capacity by the phosphomolydenum method

The phosphomolybdenum method was developed to evaluate both water-soluble and fat-soluble antioxidant capacity and used in screening samples of very different origins and compositions in search for natural sources of vitamin E and other strong antioxidants [16]. As shown by the results (Figures 2 and 3), the ethanol extract of *C. mercadoi* (CmE) has the highest total antioxidant capacity (149.91 AAE, 209.98 BHTE) while the decoction s of *M. philippica* (MpD) has the lowest (39.14 AAE, 53.99 BHTE). The total antioxidant capacity of the plant extracts expressed in both standards (AA and BHT) exhibits similar activity except for CmD that exhibits a lower BHTE as compared to MsE and just almost equal with IfD as shown in Figure 3. The top five plant extracts with antioxidant capacity expressed to AAE arranged in decreasing order are: CmE > MsD > CmD > MsE > FnE and in contrast to BHTE in the same order are: CmE > MsD > MsE > FnE > IfD / CmD. The concentrations of the plant extracts expressed as standard equivalents showed relatively strong phosphomolydenum ion-reducing activity and clearly demonstrate high antioxidant activity.

Total phenolics content

The amount of total phenolics in the selected plant extracts was determined in this study as numerous studies already reported that there is a positive correlation between the antioxidant activity potential and the amount of phenolics found in plant extracts [19-21]. As observed in the results, the amount of total phenolics varied considerably from 90.99 GAE to 570.58 GAE. Interestingly, almost all the plant extracts show significant amount of phenolics. The ethanol extract of *C. mercadoi* (CmE) was found to contain the

highest phenolics content while the ethanol extract of *M. philippica* (MpE) was found to contain the lowest phenolics content. The top five leaf extracts in decreasing order is: CmE > MsD > MsE > FnD > IfD. This result suggests that the phenolic compounds might effectively be extracted into ethanol and water (decoction) solvents.

CONCLUSION

This study was designed to evaluate the antioxidant and free radical scavenging activities of the decoction and ethanolic extracts of the leaf of selected Philippine medicinal plants; *F. nota*, *M. sagu*, *M. philippica*, *I. fagifer*, and *C. mercadoi* using two *in vitro* antioxidant assays. *C. mercadoi* leaf ethanolic (CmE) extracts showed the highest amount of total phenolics and it correlates well to its strong antioxidant against DPPH and high antioxidant capacity and or free radical scavenging activities. However, a study by Fuentes *et al.* showed that the methanolic bark extracts of *C. mercadoi* has a better IC_{50} for DPPH and higher total phenolics as compared to the leaf [22]. Phytochemical screening study on the crude methanol extract of *C. mercadoi* bark reveals the presence of saponins, condensed tannins, unsaturated lactone ring, leucoanthocyanins, and earliest chemical investigations reported sapogenin on leaves and seeds, alkaloid in the leaves, and volatile oil and safrole were also found in the leaves, bark and roots [14]. High phenolics content and strong antioxidant and free radical scavenging activity of *C. mercadoi* may be attributed to these components. Additionally, *C. mercadoi* may contain some derivatives of safrole like eugenol which is known to have strong free radical scavenging and antioxidant capacity due to the presence of phenolic groups in the aromatic ring that act as hydrogen donor to inhibit oxidation [23-25]. *M. sagu* decoction (MsD) and ethanolic (MsE) extracts are the second and third that showed highest amount of phenolics with strong free radical scavenging and antioxidant activity, respectively. This is followed by decoction extracts of *F. nota* (FnD), *I. fagifer* (IfD), *C. mercadoi* (CmD), and ethanolic extract of *I. fagifer* (IfE). In view of these, such obvious antioxidant and free radical scavenging potentials are considered to be founded by the presence of unknown antioxidants and/or by the synergistic effect of coexisting components. These findings may support their traditional/ethno-medicinal claims. To our knowledge, this is the first report on the antioxidant and free radical scavenging activities and potentials of *M. sagu, F. nota, I. fagifer* and *M. philippica.* This study further revealed that the extracts from *C. mercadoi*, *M. sagu, F. nota,* and *I. fagifer* have great potential to prevent harmful effects caused by the overproduction of radicals. The present study may provide the essential information for the utilization of these plants as a source of herbal drugs or as a source of low-cost natural antioxidants. Isolation, identification, and characterization of the inherent phytochemicals of these plants are currently in progress.

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