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RESEARCH ARTICLE

Evaluation of antioxidant activity and phytochemicals of selected methanol rattan shoot extracts from Morong, Bataan

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ABSTRACT

Background: Rattan is a member of the *Arecaceae* family grown in the tropical or subtropical climates. These plants are used as raw materials for furniture-making. In some cases, its shoots are also used as food and may possess bioactive potentials. In this study, rattan root methanolic extracts were screened for phytochemicals and evaluated for antioxidant activities.

Methodology: Six (6) methanolic extracts of selected rattan shoots were subjected to *in vitro* antioxidant assays, which include DPPH, FRAP, metal chelation, superoxide, and nitric oxide. Qualitative and quantitative phytochemical analyses were also employed.

Results: Shoots of *Calamus sp. 02* (Bangaw-Bangaw; 85.59±0.430%), *C. usitatus* (Talola; 82.45±2.215%), and *C. spinifolius* (Kurakling; 75.54±1.599%) showed the most effective DPP radical inhibition at 66.67µg/mL. Concentration-dependent reducing power (>30% scavenging at 6.64µg/mL) with no-to-low metal chelating activity was also observed in these plant food. *C. sp 02* and *C. usitatus* demonstrated an effective scavenging activity against superoxide anions at 227.3µg/mL. Nitric oxide scavenging activity was observed in all shoots with *C. merrilli* (Palasan) exhibiting highest at 78.13µg/mL. Qualitative phytochemical analyses showed that rattan shoots all contained reducing sugars, phenolics, terpenoids, and quinone compounds. Quantitative phytochemical analyses revealed that *C. sp. 02* (66.024±4.183mg GAE/g) and *C. merrilli* (1.767±0.056mg QE/g) contained the highest amounts of phenolic and flavonoids, respectively. These phytochemicals present may explain their behavior as antioxidants.

Conclusion: The study revealed that different rattan shoots showed different capacities to scavenge particular oxidants. Of these, *C. sp. 02*, *C. spinifolius*, and *C. merrilli* may be considered promising sources of natural antioxidants.

Keywords: *rattan shoots, phytochemicals, antioxidants, Calamus, Daemonorops*

Introduction

Reactive Oxygen Species (ROS) have been established to play a critical role in many pathological states such as cancers, atherosclerosis, heart disease, osteoarthritis, cataract, and diabetes [1-5]. Highly reactive ROS are produced during cellular activities either as a by-product of enzymatic reactions or as a result of intracellular metabolism of foreign compounds [6]. Increased exposure to high amounts of ROS may initiate a cascade of oxidation process and propagate organic radical species generation which may result to non-specific and irreversible alterations to biomolecular and cellular processes [7].

Antioxidants are molecules that significantly delay or inhibit the pathological effects of increased oxidative concentrations [6,8]. Though our bodies are equipped with innate antioxidant enzymes and free radical scavengers against oxygen toxicity, their levels may not be enough to combat excess levels of ROS. Thus, consumption of antioxidant-rich food may aid in the prevention of cellular damage to our bodies [6].

Fruits and vegetables are rich sources of natural antioxidants. Most of these plant food contain phytochemicals

that exhibit antioxidant properties. Phytochemicals such as flavonoids, phenolic compounds, carotenoids, and plant sterols have been reported to act on ROS by either free radical scavenging, singlet oxygen quenching, or chelating of transitional metal [6,9,10].

Rattan is a member of the *Arecaceae* family grown in the tropical or subtropical climates. Rattans are spiny and climbing palms which comprise about 600 species belonging to 13 genera, concentrated solely in the old-world tropics [11]. Four genera of rattan with 64 species are found in the Philippines, which include *Calamus, Daemonorops, Kothalsia*, and *Plectocomia* [12]. Conventionally, these plants are used for handicrafts and furniture-making. Not known to the general public, the shoots of rattan can also be used as food and may possess bioactive potentials. Thus, it is interesting to investigate the phytochemical constituents of some rattan shoots and evaluate the antioxidant activities *in vitro*. This may serve as a scientific basis for the investigation of other rattan species' potential.

Methodology

Chemicals and Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT),phenazine methosulfate (PMS), sodium nitroprusside (SNP), sulfanilamide, naphthyl ethelenediamine dihydrochloride (NED), Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na2CO3), aluminum chloride ($AlCl_3$), potassium ferricyanide [K3Fe(CN)6], quercetin, butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid (EDTA), ascorbic acid, and reduced nicotinamide adenine dinucleotide (NADH) were purchased from Sigma-Aldrich Co. St. Louis, Germany. All other chemicals used were of analytical grade.

Plant Samples

Six (6) selected rattan samples were purchased from the market of Morong, Bataan. These included five (5) *Calamus*: *C. sp. 01* (Apis), *C. sp. 02* (Bangaw-Bangaw), *C. usitatus* Blanco (Talola), *C. spinifolius* Becc. (Kurakling), *C. merrillii* Becc. (Palasan); and one (1) *Daemonorops*, *D. mollis* Blanco (Ditaan) samples. All samples gathered were authenticated by the Institute of Biology Herbarium - University of the Philippines, Diliman.

Rattan shoots were removed from its peelings, washed with distilled water, and chopped into smaller pieces. Afterwards, the samples were freeze-dried using Martin Crist Freeze dryer BETA 2-8 LSC. All dried samples were kept at 4^{o}C in sealed containers until extraction. The lyophilized rattan shoots were weighed and recorded. The percentage yield and water content of the rattan shoots were computed.

Plant Sample Extraction

Fifty grams (50 g) of each lyophilized shoots were soaked in 500 mL methanol at room temperature for 24 hours. The samples were then separated from the residue by filtration. The residue was re-extracted thrice using the same proportion. The methanol extract was concentrated using a rotary evaporator (BuchiRotavapor R-200) at 40^{o}C. Extracts were kept in tightly sealed bottles at 4^{o}C until use.

Qualitative Phytochemical Screening [13,14]

The secondary metabolites such as reducing sugars, proteins, alkaloids, glycosides, steroids and phytosterols, terpenes and terpenoids, anthraquinones, saponins, polyphenols, flavonoids, and tannins were determined in each plant sample using preliminary and confirmatory tests. The presence of reducing sugars in all plant samples was detected using Molisch, Fehling's, and Benedict's Test; proteins using ninhydrin and biuret test; alkaloids using Mayer's, Wagner's, Hager's and Dragendorff's tests; glycosides using Modified Borntranger's and Keller Killiani; steroids using Liebermann-Burchard test; terpenes and terpenoids using Salkowski's test; quinones using sulfuric acid test; anthraquinones using hydrochloric acid test; flavonoids using alkaline reagent and Shinoda's tests; polyphenols using ferric chloride test; tannins using ferric chloride and gelatin tests; and saponins using froth test.

Quantitative Phytochemical Tests

Total Phenolic Content [15]

The total phenolic content of the rattan extracts was determined using the Folin–Ciocalteu method with modifications. A volume of 15.4µL of test compounds and gallic acid standard (0-500 µg/mL) were mixed with 61.5µL of Folin Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 123µL of 7.5% sodium carbonate. The mixtures were allowed to stand at room temperature for 30 mins. The absorbances were measured at 765 nm. All determinations were performed in triplicates. The amount of phenolics was expressed as mg gallic acid equivalence per gram of the dried extract (mg/g) using gallic acid standard curve.

Total Flavonoid Content [15]

The total flavonoid content was measured using aluminum chloride colorimetric assay. One hundred microliters (100 µL) of extracts or quercetin standard solutions (0-500 µg/mL) were mixed with 2% (w/v) $AlCl_3$ (100µL) in 96-well plates. After 15 mins of incubation at room temperature, the absorbances were read at 435nm. All determinations were performed in triplicates. The amount of flavonoids was expressed as mg quercetin equivalence per gram of the dried extract (mg/g) using quercetin standard curve.

Antioxidant Assays

DPPH Scavenging Activity [16]

In order to evaluate the scavenging activity of the methanol extracts, the change of optical density of DPPH radical was monitored. Ten microliters (10µL) of standard and test compounds at different concentrations were loaded into a 96-well microplate. Afterwards, 140µL of $6.85x10^{-5}$M DPPH was added into each well. The microplate was incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517nm. Ascorbic acid (Vitamin C) was used as a standard. Measurements were taken in triplicate. DPPH scavenging capacity was calculated using the following equation:

$$\text{DPPH Scavenging (\%)} = \frac{(A_0 - A_t)}{A_t}$$

where: A_0 is the absorbance of control reaction (all reagents without test sample) and A_t is the absorbance in presence of rattan extracts or AA.

Ferric Reduction Antioxidant Power [15]

The reducing power of methanol extracts was measured using ferric reducing antioxidant power (FRAP) assay. Seventy microliters (70µL) of standard and test compounds at different concentrations were mixed with 176.5µL of 0.2M sodium phosphate buffer (pH=7.4) and 176.5µL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50ºC for 20 mins. After incubation, the reaction mixtures were acidified with 176.5µL of trichloroacetic acid (10%) and were centrifuged at 650 x g for 10 minutes. An aliquot of 273µL of the supernatant was added to 273µL of deionized water. Finally, 55µL of $FeCl_3$ (0.1%) was added to this solution. The absorbance was measured at 700 nm. Butylated hydroxytoluene (BHT) was used as standard. The test was performed in triplicates.

Metal Chelating Activity [17]

Chelation of ferrous ions was performed based on the procedure discussed. In a 96-well microplate, different concentrations of the methanol extracts (20µL) were loaded and 100µL of 0.2 mmoles/L $FeCl_2$ was added to each well. Afterwards, 40µL of 5 mmoles/L ferrozine was added. The reaction mixture was incubated at room temperature for 10 minutes. The absorbance was measured at 562nm. Ethylenediaminetetraacetic acid (EDTA) was used as the standard. Iron chelation was calculated using the equation:

$$\text{Iron chelation (\%)} = \frac{(A_0 - A_t)}{A_t}$$

where: A_0 is the absorbance of control reaction (all reagents without test sample) and A_t is the absorbance in presence of rattan extracts or EDTA.

Superoxide Anion Scavenging Activity [18]

This method was measured by the reduction of NBT according to a previously reported method. Ten microliters (10µL) of standard and test compounds at different concentrations were loaded into a 96-well microplate. Then, 100 µL 468 µM NADH, 100 µL 156 µM nitroblue tetrazolium (NBT), and 50 µL 60 µM phenazine methosulfate (PMS) were added to each well. A five-minute incubation was done at room temperature. The absorbance was measured at 560 nm. Quercetin was used as standard. Measurements were performed in triplicates. The superoxide scavenging activity of rattan shoots was calculated using the equation:

$$\text{Superoxide scavenging activity (\%)} = \frac{(A_0 - A_t)}{A_t}$$

where: A_0 is the absorbance of negative control (all reagents without test sample) and A_t is the absorbance in presence of rattan extracts or quercetin.

Nitric Oxide Scavenging Assay [15]

Griess reaction was carried out in micro scale volumes. Sodium nitroprusside (10mM, 2mL) in phosphate buffer saline was incubated with test compounds in different concentrations at room temperature for 150 minutes. After thirty minutes, 0.5 mL of the incubated solution was added

with one (1) mL of Griess reagent (0.33% sulfanilamide in 20% glacial acetic acid, 0.5mL and 0.1% NED, 1.0mL) and was incubated for 30 minutes at room temperature. The absorbance was measured at 546 nm. Gallic acid was used as standard. Measurements were performed in triplicates. The nitric oxide (NO) scavenging activity of rattan shoots was calculated using the equation:

$$\text{NO scavenging activity (\%)} = \frac{(A_0 - A_t)}{A_t}$$

where: A_0 is the absorbance of negative control (all reagents without test sample) and A_t is the absorbance in presence of rattan extracts or quercetin.

Statistical Analysis

Results are expressed as mean ± SD. The statistical analysis was performed using one-way ANOVA. The differences were considered statistically significant at $p<0.05$.

Results

DPPH Radical Scavenging Capacity

Methanol extracts of *C.sp. 02*, *C. usitatus*, and *C. spinifolius* has exhibited good concentration-dependent scavenging activity against DPP radical (Figure 1). Shoots of *C.sp.02* showed the most effective DPP radical inhibition (85.59± 0.430%), which were comparable with ascorbic acid activity (89.64±0.120%) at 66.67µg/mL. Relatively good inhibitions were also observed in *C. usitatus* (82.45±2.215%) and *C. spinifolius* (75.54±1.599%) at the same concentration. In addition, these extracts demonstrated >10% scavenging even at 16.7µg/mL dose. In contrast, low scavenging activities (DPPH inhibition < 30%) were demonstrated by *C.sp.01* even at the highest concentration. Except *C. merrillii* ($p=0.294$), all samples showed significant inhibitions compared to the negative control ($p<0.05$). Inhibitory concentrations (IC_{50}) of rattan extracts showed that *C.sp. 02* (IC_{50}=523.14µg/mL) needed lower concentration than other rattan samples in order to scavenge DPP radical by 50%. Other rattan species showed the following scavenging capacity in descending order *C.sp. 02* (IC_{50}=523.14µg/mL) > *C. usitatus* (IC_{50}=598.48µg/mL) > *C. spinifolius* (IC_{50}=681.57µg/mL) > *D. mollis* (IC_{50} =776.18µg/mL) > *C.sp.01* (IC_{50}=5341.46µg/mL) > *C. merrillii* (IC_{50}=8409.64µg/mL) extracts.

Ferric Reducing Antioxidant Power (FRAP) Assay

All rattan extracts showed concentration-dependent reducing abilities. Among these, *C. usitatus* and *C. spinofolius* were observed to have a highly significant reducing power at 53.1µg/mL by 95.04±1.380% and 91.16±0.811%, respectively, which were comparable with the reference standard, butylated hydroxytoluene (BHT) (97.90±0.185%) (Figure 2). In fact, these plant samples demonstrated no statistical difference with BHT ($p=0.99$ and $p=0.38$, respectively) and had extended their potency showing >50%

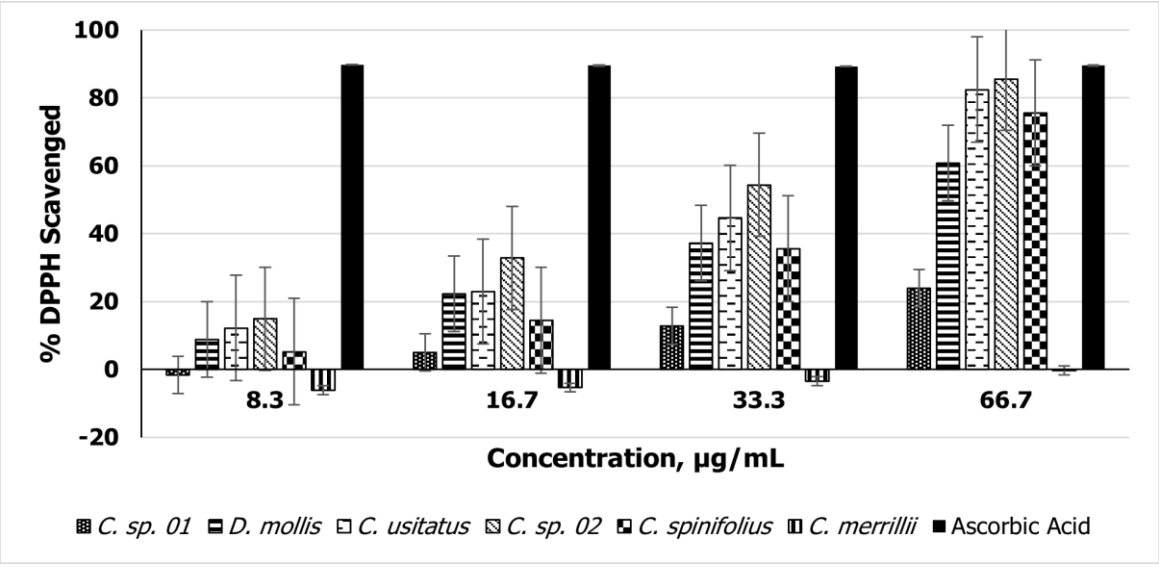

Figure 1. *DPPH scavenging activity of six methanol rattan shoot extracts at different concentrations. Each value represents mean ± SD (n = 3).*

scavenging even at 6.6µg/mL dose. The reducing ability order of the extracts was: *C. usitatus* > *C. sp. 02* > *C. spinifolius* > *D. mollis* > *C.sp.01* > *C. merrillii*.

Metal Chelation Assay

No extremely low metal chelating activity was observed from methanol rattan shoots (Figure 3). All extracts exhibited <5% iron chelation even at the highest concentration with no statistical difference with the negative control (p>0.05).

Superoxide Radical Scavenging Capacity

Methanol extracts of rattan shoots had good dose-dependent superoxide radical scavenging activity, with *C.sp. 02* exhibiting the highest effect (Figure 4). *C.sp. 02* showed the most effective superoxide inhibition (68.18±0.556%), which is comparable with quercetin activity (77.76 ±1.580 %) at 227.3µg/mL. In fact, this sample demonstrated no statistical difference with quercetin (p=0.999) at the highest concentration. Relatively good inhibitions were also observed in

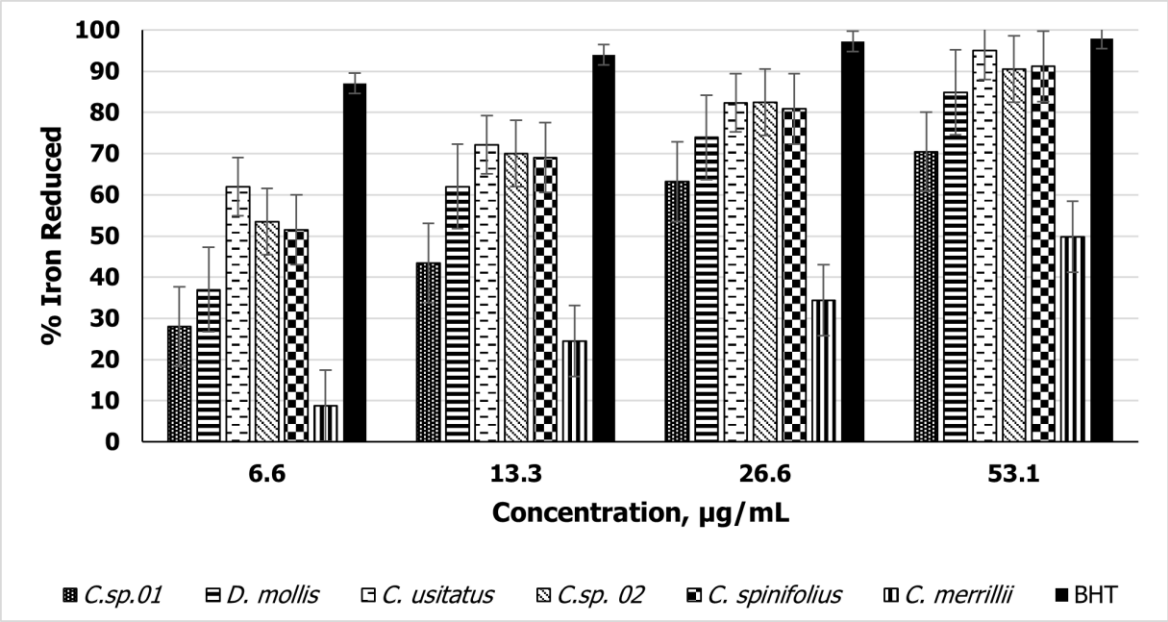

Figure 2. *Reducing power of six methanol rattan shoot extracts at different concentrations by ferric reduction antioxidant power (FRAP) method. Each value represents mean ± SD (n = 3).*

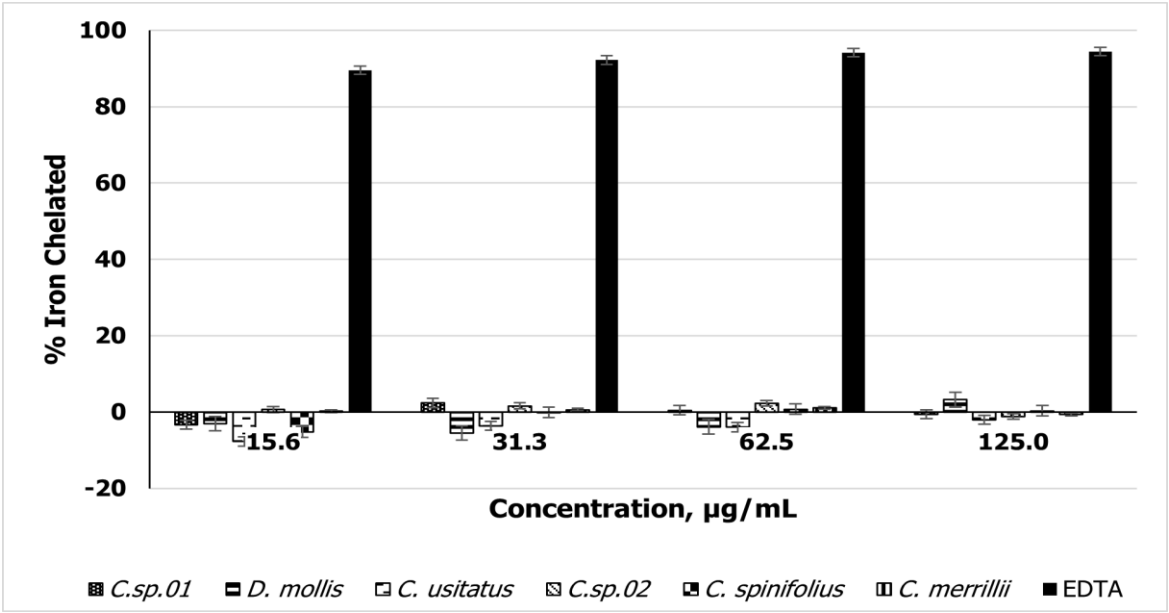

Figure 3. *Metal chelating activity of six methanol rattan shoot extracts at different concentrations. Each value represents mean ± SD (n = 3).*

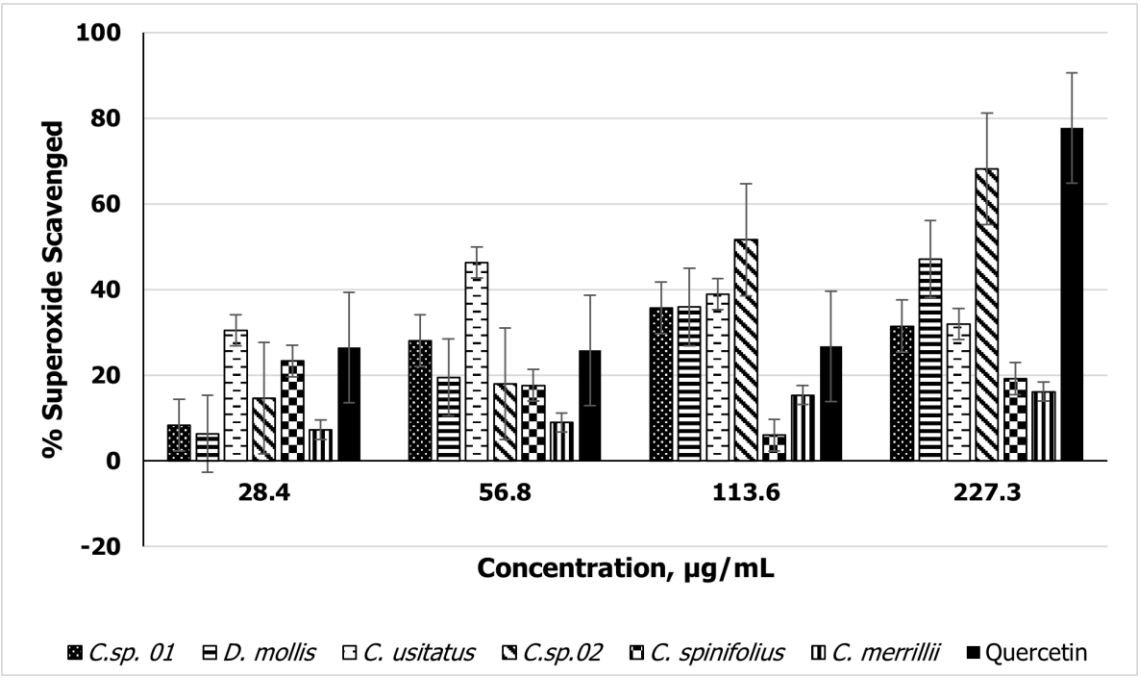

Figure 4. *Superoxide scavenging activity of six methanol rattan shoot extracts at different concentrations by the non-enzymatic phenazine methosulphate–nicotinamide adenine dinucleotide (PMS–NADH) method. Each value represents mean ± SD (n = 3).*

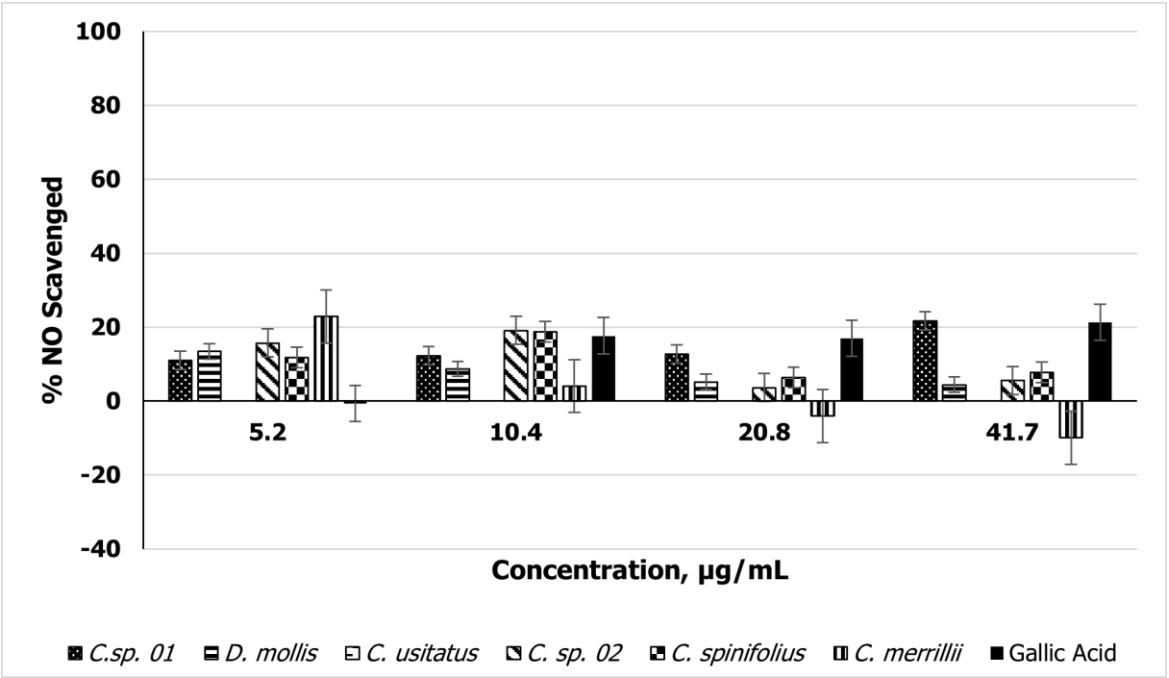

Figure 5. *Nitric oxide scavenging activity of six methanol rattan shoot extracts at different concentrations determined by the Griess-Illosvoy reaction. Each value represents mean ± SD (n = 3).*

C. usitatus (31.95±7.791%) and *D. mollis* (47.18±2.931%) at the same concentration. In contrast, *C. merrillii* demonstrated the least activity. Nevertheless, most extracts exhibited significant superoxide scavenging activity compared to the negative control ($p<0.05$). Rattan shoot extracts demonstrated inhibitory activity against superoxide in the following order: *C. merrillii* < *C. spinifolius* < *C.sp. 01* < *C. usitatus* < *D. mollis* < *C.sp.02*.

Nitric Oxide Scavenging Assay

Different nitric oxide (NO) scavenging abilities were exhibited by majority of the rattan shoots (Figure 5). *C. sp. 01* (21.75±1.596%) demonstrated the highest NO inhibitory activity at 41.7µg/mL, which is comparable to that of gallic acid standard (21.32±0.354%). In addition, it is the only

and have been used in the development of drugs [19]. Testing extracts prepared from widely utilized medicinal plants has become a rapidly growing and successful field in pharmacological science [20].

Dietary phytochemicals supplement the body's need for exogenous antioxidant systems. Antioxidants found in food combat the excess reactive oxygen species experienced by the body for human metabolism. Secondary metabolites such as phenolics, flavonoids, and anthocyanins are the major contributors to their antioxidant property [21]. Polyphenols, which include flavonoids, stilbenes, lignans, and phenolic acids, are compounds containing aromatic rings with one or more hydroxyl groups. These molecules interact with free radicals by pi-electron delocalization followed by stabilization of the aromatic nucleus through resonance. This phenomenon results in free radical chain termination [22]. Aside from this, other phytochemicals such as terpenes and terpenoids, alkaloids, and saponin compounds were also reported for antioxidant activities [23-25].

Several *in vitro* assays were performed to assess antioxidant activities of rattan shoots because antioxidant test models vary in different respects [6]. Antioxidant activity must not be based on a single antioxidant assay alone. Various assays utilize different mechanisms such as hydrogen atom transfer, single electron transfer, reducing power, and metal chelation in order to evaluate antioxidant activities of plant materials. Utilization of different reference standards for each test will provide maximal effectivity specific to the antioxidant mechanism being studied. This will allow proper comparison and evaluation of these test extracts as potential antioxidants [18,26-28].

Rattans are naturally found in the Philippine forests and are distributed throughout the country. In this study, the six (6) rattan species were chosen based on their high forest distribution [12] and constant availability in Morong market because of demand. Most Morong locals have utilized peelings for furniture-making while young shoots have been consumed as food. Edible shoots of these rattan species remain to be relatively unexplored and underutilized. Thus, this study investigated the antioxidant properties of the methanol rattan extracts to obtain more information for potential wider utilization.

One mechanism by which antioxidants inhibit oxidation is by quenching reactive species through hydrogen or electron donation [29,30]. DPPH assay is one of the most popular and frequently employed methods among antioxidant assays. Increased concentration of antioxidant compounds present may indicate an increase in the number of electrons gained, resulting in a lighter solution [31]. The variation in DPPH scavenging activity may have been caused by differences in the components and concentrations of phytochemicals in the rattan shoots. Nevertheless, it can be seen that rattan shoots have good DPPH radical scavenging activity, which can be attributed to their ability to donate protons and hydrogen atom to DPPH.

A significant indicator of a potential antioxidant activity is the determination of its reducing activity. In the FRAP Assay, the antioxidant capacity was measured through reduction of ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) by donor electrons in the sample [32]. A higher absorbance indicates a higher ferric reducing power. The observed reducing ability of rattan shoots may be attributed to the phytochemicals present that are able to donate electron with oxidants, such as metals and free radicals, converting them into stable metabolites [33].

Inorganic ions such as iron (Fe) and copper (Cu), and ultra-trace elements such as cobalt (Co), and nickel (Ni) are important in metabolism [34]. However, elevated concentrations of these transition metals may post detrimental effects through induction of oxidative damage. Transition metals can initiate hydroxyl radical production through Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\bullet$), which may increase the rate of lipid peroxidation compromising cellular integrity [35,36]. Antioxidant-rich plant samples may contain chelation abilities that may inhibit metal-catalyzed lipid peroxidation [6]. Through metal chelation assay, plant samples can be screened *in vitro*. In this test, color reduction suggests that extracts have captured ferrous ion before the formation of the red-violet Fe^{2+}/ ferrozine complex [37]. According to the results gathered, rattan extracts are not good metal chelators.

Superoxide ($O_2\bullet^-$) is a harmful free radical that may promote free radical formation of other reactive oxygen and nitrogen species, such as hydrogen peroxide, hydroxyl radicals, singlet oxygen, and nitric oxides [38]. In this assay, superoxide radical was generated in a non-enzymic system of PMS/NADPH-NBT. The $O_2\bullet^-$ generated from the reaction of NADH and phenazine methosulfate (PMS) will reduce the NBT (Nitroblue tetrazolium) to form a blue formazan [39]. A good superoxide scavenger may be helpful to lower the risk of many degenerative diseases in biological systems. The results suggest that rattan shoots contained bioactive compounds that are good scavengers of superoxide radical.

rattan shoot showing consistent inhibitions across all concentrations. Whereas *C. merrillii* (22.87±1.589%) showed highest activity among all rattan shoot extracts at 5.2 µg/mL. Most extracts exhibited significant NO scavenging activity compared to the negative control ($p<0.05$). However, some shoots were seen to behave as a prooxidant as the dose was increased to 41.7µg/mL. *C. merrillii* exhibited a significant change in NO scavenging ability from 22.87± 1.589% (at 5.2µg/mL) to -13.59 ± -9.945% (at 41.7µg/mL). The same pattern of activity was demonstrated by other rattan shoots.

Phytochemical Analysis

Qualitative phytochemical analyses showed that all rattan shoots contained reducing sugars, coumarins, phenolics, terpenoids, and quinone compounds. Steroids and phytosterols were only present in *D. mollis* and *C. sp. 02*. Only *C. sp. 01* contained saponin and lacked flavonoid compounds. Anthraquinones, tannins, proteins and alkaloids were not present among all rattan shoots tested. The total phenolic content (TPC) and total flavonoid content (TFC) of rattan shoots were determined using the Folin-Ciocalteu and aluminum chloride methods, respectively. The TPC values in the methanol rattan shoot extracts analyzed were in the range of 8.154 – 66.02 mg gallic acid equivalents (GAE) per gram of dried extract. Among the rattan shoot analyzed, *C. sp. 02* revealed the highest TPC at 66.02±4.183 mg GAE/g. On the other hand, *C. merrillii* (1.767±0.056 mg quercetin equivalents (QE)/g) contained the highest TFC while *C. sp. 01* (0.803±0.022 mg QE/g) being the least.

Discussion

Nowadays, plants are important sources of medicines, especially in developing countries that still use plant-based traditional medicine for healthcare. Several studies have shown that the bioactive constituents of ethno-medicinal plants are sources of curative actions for certain diseases

Table 1. *Qualitative analysis of methanolic extracts of selected rattan shoots*

Rattan Shoots	Flavonoids	Coumarins	Reducing Sugars	Proteins	Saponins	Glycosides	Alkaloids	Terpenes/ Terpenoids	Tannins	Phenolics	Steroids and Phytosterol	Quinones	Anthraquinones
C. sp. 01	-	+	+	-	+	+	-	+	-	+	-	+	-
D. mollis	+	+	+	-	-	+	-	+	-	+	+	+	-
C. usitatus	+	+	+	-	-	+	-	+	-	+	-	+	-
C. sp. 02	+	+	+	-	-	+	-	+	-	+	+	+	-
C. spinifolius	+	+	+	-	-	-	-	+	-	+	-	+	-
C. merrillii	+	+	+	-	-	+	-	+	-	+	-	+	-

Legend: '+' represents presence of the phytoconstituent; '-'represents absence of the phytoconstituent

Table 2. *Quantitative analysis of methanolic extracts of selected rattan shoots*

Rattan Shoots	FlavonoidsTotal Phenolic Content (mg GAE/g dried extract)	Total Flavonoid Content (mg QE/g dried extract)
C. sp. 01	21.04±1.349	0.803±0.022
D. mollis	53.30±2.934	1.280±0.046
C. usitatus	62.81±3.774	1.744±0.104
C. sp. 02	66.02±4.183	1.262±0.190
C. spinifolius	29.24±1.521	1.619±0.005
C. merrillii	8.154±0.370	1.767±0.056

Abbreviations: GAE – gallic acid equivalents; QE – quercetin equivalents

Nitric oxide (NO) is a highly reactive compound that mediates several physiological and pathological processes. At lower concentrations, NO plays vital roles in vasodilation, blood pressure regulation and cellular signaling [40,41]. However, NO as a free radical, readily reacts with other radical species (*e.g.* superoxide [$O_2\bullet^-$], lipid alkoxyl [LO•], and peroxyl [LOO•] radicals) which damages a wide array of molecules in cells including DNA, proteins, sugars, and lipids [42]. Scavengers of nitric oxide found in plant food compete with oxygen, leading to reduced production of nitric oxide. Differences in the nitric oxide scavenging abilities exhibited by majority of rattan shoots may be attributed to the phytochemicals behaving as pro-oxidant molecules. This behavior may be due to variation in the phytochemical conditions such as metal ion concentration, pH, and oxygen presence [21,43,44]. Polyphenols with low oxidation potentials (Epa) exhibit antioxidant activity, while those with high Epa values act as pro-oxidants [6].

Similar findings have been reported on the antioxidant potentials of different rattan species. Shoots of *Plectocomia himalayana* showed a relatively good DPPH radical scavenging activity comparable to that of BHT and ascorbic acid standards [45]. Methanol extract of *Calamus tenuis* RoxB showed dose-dependent inhibition of DPPH radical with IC_{50} value of 169.50µg/mL [46]. Three *Calamus* species namely, *C. manan*, *C. caesius*, and *C. ornatus*, exhibited strong activities against DPPH radical with IC_{50} values ranging from 8.80 to 170.19 µg/mL [47].

Isolated compounds from rattan species have been reported to combat oxidative stress. Dragonins A and B, which are flavan-3-ol-dihydroretrochalcone derivatives from *Daemonorops draco* resin inhibited superoxide anion production with IC_{50} value = 4.5 ± 0.8 µM, respectively [48]. Quiquelignans found in *Calamus quiquesetinervius* stems showed radical scavenging activities against nitric oxide, hydroxyl radical (•OH), and super oxide anions (O_2^-) [49]. While quiquesetinerviusides, a phenylpropanoid glycoside derivative from the same plant species, possesses weak DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, it has potent •OH radical scavenging activity (IC_{50} 3.6-8.4 µM) [50].

Conclusion

In conclusion, the study revealed that rattan shoots, which contain adequate amounts of secondary metabolites such as phenolics and flavonoid components, exhibited high antioxidant, reducing, and free radical scavenging activities. Various phytochemical components within these rattan shoots provided the differences in the degree of antioxidant capacity against particular oxidants. *Calamus sp. 02*, *C. spinifolius*, and *C. merrillii* for example, possessed higher or equal antioxidant activity as compared to known standards. Through the *in vitro* tests, these plant extracts may be potential sources of natural antioxidants, which may be beneficial in the inhibition of various oxidative stresses. However, the specific component/s in these plant extracts remain unclear. Therefore, isolation and characterization of the antioxidant compounds in these plant sources are deemed necessary. Also, *in vivo* antioxidant activity may be explored to determine its effects in a biological system.

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