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IDENTIFICATION AND BIOACTIVITY PROFILINGOF SELECT FERNS FROM MT. MINGAN, GABALDON, NUEVA ECIJA, PHILIPPINES

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ABSTRACT

Some ferns are utilized as food and medicine in the Philippines. Five species were collected from Mt. Mingan, Gabaldon, Nueva Ecija and were identified and screened for their bioactive constituents and radical scavenging activity. These include *Nephrolepis exaltata, Tectaria crenata*, *Davallia solida*, *Christella dentata*, and *Selaginella cf. cuppressina*. All plant samples contain fatty acids, sugars, coumarins, anthrones, and alkaloids. *N. exaltata* has triterpenes, saponins, and anthraquinones whereas both *T. crenata* and *D. solida* contain tannins, triterpenes, phenols, saponins, flavonoids, and anthraquinones.*S. cuppressina* has essential oils, triterpenes, phenols, and flavonoids while *C. dentata* contain essential oils, tannins, saponins, and anthraquinones. The extract of *D. sollida*exhibited the highest scavenging activity of 76.68% against DPPH free radicalswhile *N. exaltata* extract showed the lowest activity (61.88%). These ferns are therefore considered as natural sources of bioactive compounds with effective antioxidant activities.

Keywords: Ferns, phytochemical screening, DPPH radical scavenging activity, functionality profiling

INTRODUCTION

Mt. Mingan in Gabaldon, Nueva Ecija, Philippines is a haven of diverse flora and fauna. One of the most abundant plants in the area is fern. Some ferns are utilized as food and natural medicine by the people inhabiting nearby. As food, fern shoots are prepared as vegetable salad, and mixed with fish soup and other vegetables. Moreover, it is utilized in folkloric medicine for inflammation, pain, wounds, insect bites, and skin problems.With these remarkable uses of ferns in the community near Mt. Mingan, it is indeed necessary to evaluate their bioactivities in order to establish their roles in nutraceutical and pharmaceutical industries. In recent work, methanolic extracts of three ferns (*Drynaria quercifolia, Microsorum punctatum* and *Pyrrosia adnascens)* collected from Bukidnon, Philippines are found to containalkaloids, anthraquinones, phenolics, saponins, tannins and terpenoids which support their biological activities such as antioxidant and anti-inflammatory**[1]**.

Phytochemicalsare biologically active, non-essential chemical compoundsabundant in plants that have health protective benefitsin humans **[2]**. These secondary metabolites are chemicals not necessary for the plant's immediate survival but are synthesized for increased plant fitness and protection by allowing it to interact with its environment **[3]**.Saxena *et al.* **[4]** classified these bioactive and disease preventing phytochemicals into NSA (non-starch polysaccharides), antibacterial and antifungal, anticancer, detoxifying agents, antioxidants and others. The largest and ubiquitous phytoconstituents which makes up 45% of the plant phytochemicals is phenolics **[4]**. They are reported as the main source of antioxidant activity **[5]** due to their redox properties which let them act as reducing agents, hydrogen donors and single oxygen quenchers **[6]**. Antioxidant capacity of phenols is attributed in its ability to chelate metals ions involved in free radical production**[7]** and its capacity to inhibit some enzymes involved in radical generation**[8,9]**.

In this study, five ferns were identified and profiled their phytochemicals and antioxidant properties in our intention to popularize their utilization and establish their function as natural forest products.

MATERIALS AND METHODS

Collection of Ferns

The five ferns were collected from Mt. Mingan, Gabaldon, Nueva Ecija, Philippines.Plant samples were placed in plastic bags and brought to the laboratory for

identification and air-dried for 5 days for extraction.

Identification of Ferns

The collected ferns were identified based on the morphological characteristics.The structures and arrangement of leaves or fronds, the size and shape of spores, the size, shape and orientation of rhizome, and the appearance of the stipe were determined and recorded. Each fern was described based on these parameters.

Extraction

The air-dried samples were pulverized using a food processor. Ten grams of each samplewas soaked in 600 ml of 95% ethanol for 2 days. After soaking, extracts were filtered using Whatman filter paper No. 2 to separate the plant powder. Filtrates were concentrated in a rotary evaporator up to dryness. The extract yields were determined and subjected to phytochemical screening and antioxidant assay.

Phytochemical Screening

Phytochemical screening was carried out to detect secondary metabolites present. Each extract was spotted on marked and labeled TLC (Thin Layer Chromatography) 7 x 4 cm, and was developed in acetate-methanol (7:3) mixture in the developing chamber. The spots for certain metabolite were visualized on the TLC plates and were exposed under UV light and hot plate to check the separation of the different compounds.For typical visualization of secondary metabolites, vanillin-sulfuric acid reagents were used. This solution determines the presence of phenols, sterols, triterpenes, and essential oils. Methanolic potassium hydroxide was used to test anthraquinones, coumarins and anthrones while phenolic compounds and tannins were detected through the use of potassium ferricyanide-ferric chloride reagent. Dragendorff's reagent was utilized to spot alkaloids and antimony (III) was used to detect the presence of flavonoids **[10]**.

DPPH Radical Scavenging Assay

The concentrated extract was used to make a stock solution and aliquot was taken to make 1000ppm dilution and 1000ppm of catechin as control (1mg/mL). One ml of prepared stock solution was mixed with four mL of 0.1 mM DPPH solution in separate plastic cuvette. Reactions were done in triplicate. The prepared mixtures were incubated in the dark at 37ºC for 30 minutes. The absorbance readings were monitored at 517 nm using a UV VIS spectrophotometer. A lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical scavenging activities were compared to the activity of the control catechin. The

ability to scavenge the DPPH radical was calculated using the formula: (A0-A1)/A0] × 100, where A0 was the absorbance of the control which is the DPPH without the test sample and A1 was the absorbance of the test sample containing the mixture of the DPPH and the sample**[11]**.

RESULTS AND DISCUSSION

Identificationand Description of Ferns

Ferns are one of the most abundant and important plants in Mt. Mingan, Gabaldon, Nueva Ecija. However, there is a need to identify this diverse groupin order to popularize and establish their full utilization. Five ferns were identified based on their morphological structures. These include *N. exaltata*, *T. crenata*, *D. solida*, *C. dentata*, and *S. cuppressina* (Figure 1).

*N. exaltata*rhizome is sub-erect and short with upright and sterile dagger shape fronds that hangs downward and grows in masses at maturity. Its pinna is compound unipinnate that have linear leaflets with acute apex, flat and smooth lamina with white hairs on the surface, and entirely to slightly toothed margins. Sori are located at the lower surface of the pinna, arranged in two rows at the margins of each pinna. Stipe is glossy brown and is densely covered with golden brown hair-like structures. This fern was found in abundant number beside the stream and along the paths of Mt. Mingan.

The rhizome of *T. crenata*is short and erect with lamina rough in texture and is densely covered with sori. Herbaceous color green on the upper surface and paler beneath. It has four pairs of pinna that are oblong – lanceolate, imparipinnate with terminal pinna pinnatifid and margin undulate. Pinna located at the base is the largest gradually decreasing its size towards the terminal pinna. Apex is caudate that gradually narrows at the base. Main veins are distinct and venation is reticulated. Stipe and axis is smooth and glossy olive green. It is found in the shady area attached on the mountain slopes of Mt. Mingan.

D. solida was found on an exposed place and deeply shaded area growing on a soil thickly covered with leaf litters. It has fully exposed rhizomes that are hairy, thus,its common name is hare's root fern. Stipe adaxially grooved that becomes narrow toward the apex, dark green, smooth; some parts are covered with hairs on the junction of the rachis and petiolule. Lamina is smooth and leathery; tripinnate from the middle towards the base; pinna is linear triangular with apex acuminate and margin dentate and pinnules lobed. Veins are longitudinal reaching the

margin. Sori are located on each lobes containing brown spores.

C. dentata often called as downy maiden fern was collected at the river banks of Mt. Mingan attached to the soil. It is bipinnate with pale brown stipe and rachis covered with small white hairs. Its pinna rachis is pale green and smooth. Its pinna is yellow green with dentate margin and with pinna leaflets pinnate. Sori are orange and were scattered all over the lower surface of the leaves.

S. cuppressina is found attached on rocks and on the forest floor usually moist and shady. They are spike mosses or lesser club mosses belonging to fern allies. Stem is sub-erect and leaves are evergreen, thin, lanceolate and tripinnate scale-like (microphylls) bearing ligules with pointed apex. Leaves are dimorphic. It is relatively small with sporophyte measuring approximately 6.5 cm.

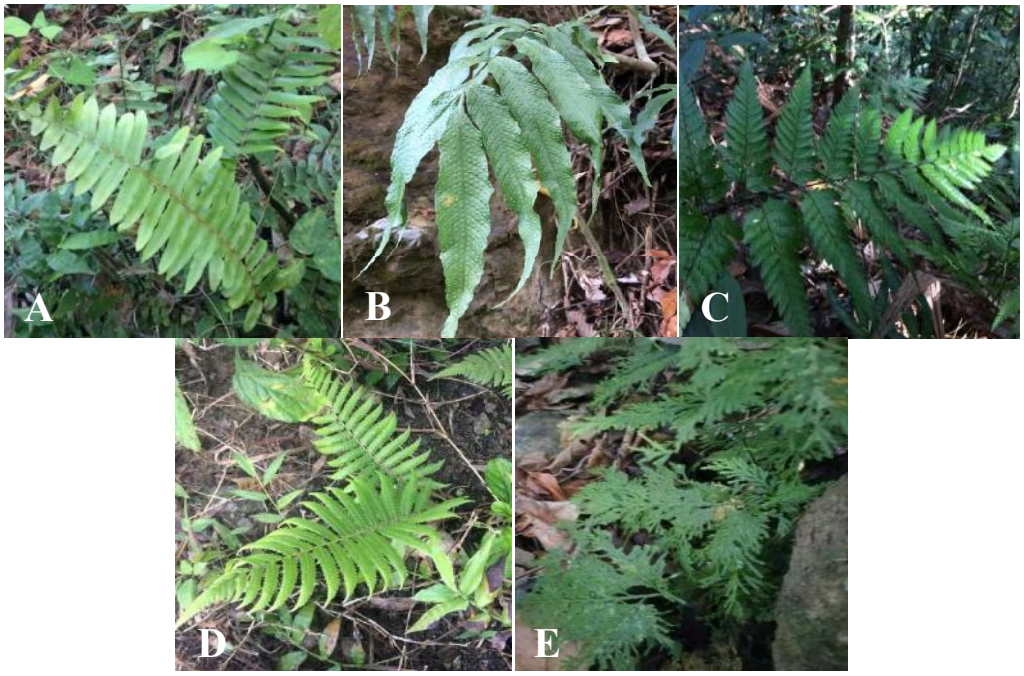

Figure 1: The five collected ferns: (A) *N. exaltata*, (B) *T.crenata*, (C) *D. solida*, (D) *C. dentata*,and (E) *S. cuppressina*

Phytochemical Compositions

Phytochemicals are substances or compounds derived from plants which play significant roles in preventing or controlling various diseases in human.The phytochemical constituents of the five ferns were also elucidated in the present study. The results of phychemical screening are shown in Table 1. Interestingly, all plant samples contain fatty acids, sugars, coumarins, anthrones, and alkaloids. *N. exaltata* has

triterpenes,saponins, and anthraquinones whereas both *T. crenata* and *D. solida* contain tannins, triterpenes, phenols, saponins, flavonoids, and anthraquinones.*S. cuppressina* has essential oils, triterpenes, phenols, and flavonoids while *C. dentata* contain essential oils, tannins, saponins, and anthraquinones.

Several studies reported the phytochemical constituents and functional activities of the five ferns. *N. exaltata*has essential oils which are obtained by performing hydro-distillation. These essential oils have a potential antibacterial and antifungal activity against various microorganisms. In addition, *N. exaltata* oil showed cytotoxicity in breast, colon and lung carcinoma **[12]**.Rachdiati and Zakariya **[13]** had surveyed a fern species under the genus *Tectaria. T. singaporeana* looks similar with *T. crenata* but this species has a broader leaf width. This fern it contains the compounds like alkaloids, phenols and saponins. This species of fern has medicinal use; it is prepared by boiling the roots with water and drinking it once a day to relieve back pain.The genus *Selaginella* is a rich source of biflavonoids, flavonoids, chromone glycosides, and phenolic constituents **[14-16]**. Other species like *S. inaequalifolia* shows the presence steroids, triterpenes, phenolic group, tannin, sugars and catechin **[17]**. Phytochemical screening of *S. btyopteris* revealed that it contains alkaloids, flavonoids, steroids, saponins, cardiac glycosides and tannins however the presence of anthraquinones is negative **[18]**.Many species of the genus *Davallia* including *D. solida* has a total of more than 80 compounds like triterpenoids , sesquiterpenoid, flavanones, flavan-3-ols, xanthones and cyanogenic glycoside, and proanthocyanidins **[19]**.*D. solida* has been used as medicine, its rhizome is used as an herb tonic to treat osteoporosis, arthralgia, and arthritis **[19]**. Finally, acetone extract of *C. dentata* showed the presence of phenols, xanthoproteins and carbohydrates, the chloroform extract showed five chemicals namely phenols, saponins, tannins, carboxylic acids and carbohydrates **[20]**.

Radical Scavenging Activity

The most common method to determine the antioxidant activity is the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay **[21]**. The radical scavenging activities of ethanolic extracts of the five ferns were analyzed (Table 2). Apparently, all extracts exhibited radical scavenging activities and they vary depending on the species. The *D. solida* extract had the highest radical scavenging activity of 76.78% followed by *T. crenata, S.*

cuppressina, C. dentata and N. exaltata (73.92%, 71.05%, 68.76% and 61.88%, respectively). Although these values are lower than the cathechin, it is important to note that the five ferns could be valuable resources of antioxidants. In the previous work, Valizadeh *et al*. **[22]** reported the antioxidantpotentials of the eight species of ferns. Moreover, highland ferns are also reported to be potential sources of antioxidant and can also serve as an antibacterial agents and glucosidase inhibitor **[23]**.

Table 1: Phytochemical compositions of the five ferns

Phytochemical	*N. exaltata*	*T. crenata*	*S. cuppressina*	*D. solida*	*C. dentata*
Fatty acids	+	+	+	+	+
Sugars	+	+	+	+	+
Triterpenes	+	+	+	+	-
Coumarins	+	+	+	+	+
Saponins	+	+	-	+	+
Anthraquinones	+	+	-	+	+
Anthrones	+	+	+	+	+
Alkaloids	+	+	+	+	+
Tannins	-	+	-	+	+
Phenols	-	+	+	+	-
Flavonoids	-	+	+	+	-
Essential oils	-	-	+	-	+

Table 2: DPPH radical scavenging activity of the ethanolic extracts of five ferns

Fern Ethanolic Extract	Radical Scavenging Activity (%)
N. exaltata	**61.88**
T. crenata	**73.92**
S. cuppressina	**71.05**
D. solida	**76.78**
C. dentata	**68.76**
Catechin (control)	**82.23**

CONCLUSION

Five species of ferns were collected from Mt. Mingan, Gabaldon, Nueva Ecija Philippines and were identified as *N. exaltata*, *T. crenata, S. cuppressina, D. solida,* and *C, dentata.*Each fern has unique phytochemical constituents and different radical scavenging activities.

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