



Journal Homepage: - www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/3873
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/3873>



RESEARCH ARTICLE

BRINGING BACK THE LOST VALUE OF PHILIPPINE EDIBLE FERNS: THEIR ANTIOXIDANT, PROTEINS AND UTILIZATION.

*Victor B. Amoroso^{1,2}, Rainear A. Mendez^{1,3} and Annabelle P. Villalobos⁴.

1. Center for Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University, Musuan, Bukidnon 8710 Philippines.
2. Department of Biology, Central Mindanao University, Musuan, Bukidnon 8710 Philippines.
3. Department of Chemistry, Central Mindanao University, Musuan, Bukidnon 8710 Philippines.
4. Department of Science and Technology Balik-Scientist and Biotech Drug Discovery and Development Consultant (USA).

Manuscript Info

Manuscript History

Received: 15 February 2017
 Final Accepted: 11 March 2017
 Published: April 2017

Key words:-

Pteridogarden, antioxidant, proteins, phytochemicals, fern recipe

Abstract

Ferns have myriad compounds and are used by native people as food for a long time but this use seems to diminish over time. Knowing their use as food, bioactives and proteins were determined to demonstrate their health and wellness benefits. Ten species of edible ferns were collected and propagated in the Pteridogarden. The young sterile fronds were used to determine bioactives and proteins. The bioactives were determined by thin layer chromatography, DPPH assay, Bradford assay and SDS-PAGE. A seminar-field visit was conducted to disseminate the new information gathered and a recipe book was prepared. Phytochemical analysis revealed alkaloids, saponins, phenolics, terpenes and flavonoids. The antioxidant activity ranged from 32.5-76.0% relative to ascorbic acid or 31-152 μ mol ascorbic acid/g wet weight. The protein content ranged from 0.1-4.4mg/g wet weight. The molecular weights of component proteins ranged from 10-250kDa. *Marsilea crenata*, *Acrostichum aureum*, *Pteris ensiformis*, *Angiopteris palmiformis*, *Asplenium nidus* and *Diplazium esculentum* exhibited high quantifiable antioxidant activity. On the other hand, *M. crenata*, *A. aureum*, *P. ensiformis*, *A. palmiformis*, *D. esculentum* and *Stenochlaena palustris* have high protein content. These results together with food recipes were disseminated to the public. The ten species of edible ferns contain quantifiable amounts of antioxidants and proteins. Based on these data and the dishes prepared, *M. crenata*, *D. esculentum*, *S. palustris*, *P. ensiformis*, *A. palmiformis*, *A. nidus* and *A. aureum* are potential and alternatively good food source.

Copy Right, IJAR, 2017,. All rights reserved.

Introduction:-

The use of indigenous plants as source of food and medicine dates back since the beginning of mankind. The main staple food in the Philippines is traditionally starch-based and this has been recognized by the Food and Agriculture Organization (FAO) as one of the causes of the protein deficiency in the populace. Many Filipinos eat vegetables but they seem to choose only those that are palatable to them probably because of inadequate science-based knowledge

Corresponding Author:- Victor B. Amoroso.

Address:- Center for Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University, Musuan, Bukidnon 8710 Philippines.

on their nutritional values. On the other hand Filipinos adhere to meat, dairy or plant seeds as source of proteins and not from leafy vegetable. With fast paced society, advanced technologies on food preparation techniques, somehow, we developed a habit of relying on fast foods instead of home cooking. This practice led to the under-exploitation of a wide range of vegetables most especially local and indigenous vegetable materials. As reported by Cai et al. (2004) and Hafidh et al. (2009), many indigenous foods contain antioxidants which are important in the prevention of cancer and other degenerative diseases by acting as free radical scavengers. One of these indigenous plants are ferns which have been used as food and herbal medicines for a long time (de Winter and Amoroso, 2003; Benjamin and Manickam, 2007).

The Philippines is one of the mega-biodiversity countries. It holds 11% of the 9,000 species of pteridophytes worldwide even though the Philippines occupies a measly 0.2% of the world's total land area (Smith et al., 2006; Amoroso et al., 2009; Amoroso et al., 2011). Of these pteridophytes, more than a hundred species are economically important with 24 species in the Philippines reported to be edible (de Winter and Amoroso, 2003).

The functional properties of ferns that are beneficial to human health is their antioxidant activities (Shin and Lee, 2010). There is a relationship between consumption of antioxidant-rich foods and prevention of human disease (Rathore et al., 2011). It is also known that the bioactive components of ferns are the phenolics, flavonoids, alkaloids and terpenoid families (Ho et al., 2010) in which the phenolics and flavonoids have been demonstrated to be potent antioxidants (Dai and Mumper, 2010 and Procházková et al., 2011). From our previous study, we reported 10 indigenous edible ferns found in Mindanao, Philippines (Amoroso, 1990). These edible species which are also medicinal ferns are: *Diplazium esculentum* Retz. Sw., *Marsilea crenata* Presl., *Ceratopteris thalictroides* (L.) Brongn., *Pteris ensiformis* L., *Acrostichum aureum* L., *Stenochlaena palustris* (Burm. f.) Bedd., *Pteridium aquilinum* L. Kuhn, *Asplenium nidus* L., *Angiopteris palmiformis* (Cav.) Christ., and *Sphaeropteris glauca* (Blum) R. M. Tryon. Some of these ferns grow abundantly in Mindanao and are used by the local people of Mindanao as food and medicine up to the present time. Like in any developing countries, these ferns are utilized by the poor villagers as the low cost functional foods. We have examined the taxonomy, morphology and ecology of these edible ferns. Furthermore, these ferns showed the presence of active principles such as alkaloids, tannins, glycosides, saponins and organic acids (Amoroso, 2013) but no data has been reported on their antioxidant activity, protein content, and relative component proteins.

In the study by Chai et al. (2012), the edible young sterile frond of *S. palustris* has higher antioxidant activity than the mature fertile frond and young fertile frond. The young sterile frond also contained 20 fold more anthocyanins than any of the mature fronds and that the total polyphenols correlated with radical scavenging activity. In Canada and Northeastern America, the fiddleheads of *P. aquilinum* and *Matteuccia struthiopteris* have antioxidant activity twice that of blueberries, which is claimed to have high antioxidant activity among fruits. *P. aquilinum* is also found in the Philippines. Some ferns are also a good source of niacin, potassium, phosphorus, and high in iron and fiber (Marañon, 1935; Afriastini, 2003) but low in sodium. They have also been reported to be effectively used as natural cosmetic ingredients for healing skin, skin smoothening, and protection against aging or UV damage (Tanaka et al., 2004; Jin et al., 2005; Kim et al., 2006; Parvez et al., 2006).

All these data suggest that some species of ferns could be potent source of antioxidants, which are protective against the risk of chronic diseases by reactive oxygen species (free radicals). These also point to possible health benefits associated with the consumption of ferns as vegetables. Because of protein deficiency in the Filipino diet as well as high incidence of cancer and other degenerative diseases, it is therefore worth re-introducing ferns as part of the Filipino diet. This study was conducted to determine the antioxidants and proteins of the ten indigenous edible species of ferns. The results of the study will be used to educate and convince the public of the health benefits and nutritional values of these indigenous edible ferns.

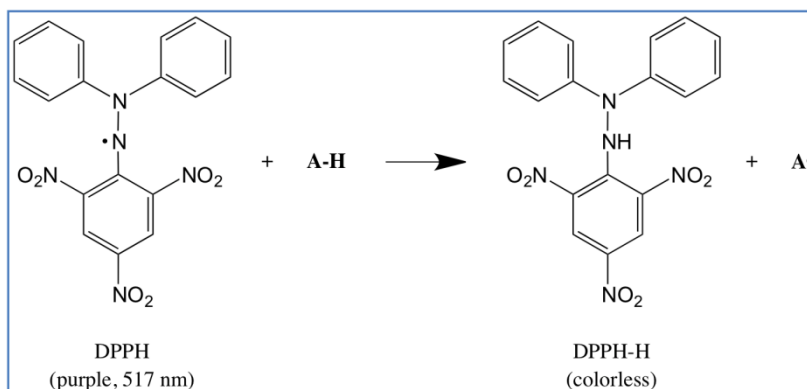
Materials And Methods:-

Propagation of Edible Ferns in the Pteridogarden in Mt. Musuan Botanical Garden, Central Mindanao University (CMU), Bukidnon, Philippines – We established a pteridogarden (garden for edible and medicinal ferns) at the Mt. Musuan Botanical Garden from wildlings of ten indigenous edible species collected throughout Mindanao Island, Philippines (Figs. 1 and 2). After collection, the plants were segregated and planted based on their individual requirements for water, for sunlight, and the needed distance between plants of the same species. This pteridogarden is a form of *ex-situ* conservation and will not deplete the wild population of the ten species of edible ferns.

At the time the plants become mature, young sterile tender fronds were harvested, processed and tested for antioxidants, phytochemical screening, total proteins and relative component proteins. The young sterile tender fronds were also used in food preparation as fern gourmets.

Preliminary test for antioxidant by the ferric chloride test – In this study, a preliminary test was made for the antioxidant activity in each species of fern by the ferric chloride (FeCl_3) test. The FeCl_3 test is a general test to determine the presence or absence of phenols in a given sample. Compounds with phenolic group will form a blue, violet, purple, green or red-brown color upon addition of aqueous ferric chloride. Since most antioxidants contain phenols, the FeCl_3 test serves as a quick preliminary screening test for antioxidants in field samples (Trease and Evans, 2002). In this study, fern fronds were added with boiling water, immediately centrifuged, and 250 μL of the supernatants were each added with a drop of 10% FeCl_3 (Scharlau, Elmar Marketing, Iligan, Philippines) solution. Results were evaluated by visual inspection (Usman et al., 2009).

Free Radical Scavenging Activity of the Methanol/Aqueous Extract by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay – We determined the free radical scavenging activity of the ten species of edible ferns by the DPPH assay and compared it against L-ascorbic acid as standard. DPPH assay is an easy and accurate method of determining the level of antioxidants in vegetables and food extracts (Huang et al., 2005). It is usually used to test the ability of compounds to act as free radical scavengers or to evaluate the ability of food to donate a hydrogen atom. The assay involves the use of DPPH, a stable free radical that has high absorption at 517nm. When the odd electrons of DPPH pair off with the electrons from phenolic compounds, the absorbance decreases. The degree of decolorization is a measure of the reducing capacity of a substance and is evaluated as the antioxidant activity (Silva et al., 2002). The DPPH method is not specific to any antioxidant component but applies to the overall properties of the sample (Prakash et al, 2000). In this study, we used ascorbic acid as the reference standard. Ascorbic acid is known to scavenge the free radical in DPPH, which is observed as a complete decolorization of DPPH from purple to colorless [refer to reaction scheme below (Pyrzynska and Pękal, 2013)].



We harvested the young sterile tender fronds, washed to remove dirt and debris, weighed, air-dried and mechanically ground into homogeneous fine pieces. The ground samples were soaked in methanol for 48 hours, filtered to obtain the methanol extract and subsequently concentrated by rotary evaporation at 40°C.

The concentrated methanol extracts were tested for antioxidant activity by the DPPH assay and for phytochemical screening. For the DPPH assay, we followed the method of Mosquera (2007) with some modifications. We developed a 96-microtiter configuration using the 96-well plates (Corning Inc., Corning, NY, USA). The concentrated extract was dissolved in 15:5:2 solvent mixture of dimethyl sulfoxide [(DMSO) Sigma Aldrich, St. Louis, Missouri, USA], methanol (Scharlau, Elmar Marketing, Iligan, Philippines) and de-ionized water up to a final concentration of 2mg/mL. This sample was added to the freshly prepared DPPH (Sigma Aldrich, St. Louis, Missouri, USA) solution and the reaction mixture was incubated in the dark since DPPH is light sensitive. Thirty (30) minutes after the addition of the sample extract, the decolorization of the DPPH solution in the presence of a free radical scavenger in the sample extract was measured at 517nm using Spectramax 250 spectrophotometer (Molecular Devices®, Sunnyvale, California, USA). The 96-well plate was automatically shaken to mix the solution prior to reading the Absorbance (A) at 517nm. L-ascorbic acid (Sigma Aldrich, St. Louis, Missouri, USA) and DPPH solutions were tested in parallel to serve as positive and negative controls, respectively. In this test, the result is expressed as micromole antioxidant equivalent per 100 gram of sample, which is compared with that of L-

ascorbic acid as standard. Scavenging activities were measured using this formula:

$$\% \text{ Activity} = [(A_{\text{Control}} - A_{\text{Extract or difference}}) / A_{\text{Control}}] \times 100\%$$

Phytochemical Screening by TLC– The organic components of the aqueous extracts of the young sterile tender fronds were determined by TLC. The tests for the presence of alkaloids, phenolic compounds, saponins, terpenes and flavonoids were performed. Commercially available TLC plates (Analtech, Newark, Delaware, USA) were used to separate organic compounds present in the solvent extract. Using fine capillary tubes, a spot on the TLC plate at least 2cm from the edge were made with the extract. The chromatogram was developed in a chamber using a standard method. The dried TLC plates were visualized by Dragendorff solution (alkaloids), acetic anhydride-sulfuric acid chloride (saponins), potassium ferricyanide-ferric chloride (phenolics), Antimony (III) chloride (terpenes) and vanillin/sulfuric acid (flavonoids).

Protein Content by the Bradford Assay– Crude extracts from the ten species of indigenous edible ferns were obtained using 0.1N NaOH (Scharlau, Elmar Marketing, Iligan, Philippines). These extracts were tested for total protein content (Bradford M., 1976) with slight modification. The method does not require that the protein is enzymatically active that extraction of protein can be attained with 0.1N NaOH. This efficient extraction of protein in fern frond is suggested by Essuman et al. (2014). In this study, the method is a microassay in a 96-microtiter well configuration using 96-well plates. Absorbance was measured at 595nm and determined using the Molecular Devices® Spectramax 250 spectrophotometer.

The assay used Bovine Serum Albumin (BSA) as standard (Thermo Scientific, Rockford, IL 61101 USA), which is run at different concentrations ranging from 50 to 75µg/mL in parallel with the samples. The protein concentration in the indigenous fern extracts was determined by interpolating their absorbance values against the BSA standard curve.

Relative Component Proteins by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)–

The proteins from the ten species of indigenous edible fern samples were extracted using the Laemmli sample load buffer (Bio-Rad Laboratories, Hercules, California, USA). The fern sample-Laemmli sample load buffer mixture were set aside at room temperature for 30 minutes, centrifuged and supernatants collected. Fifteen microliters of the supernatant samples were each loaded into wells of 10% laboratory casted polyacrylamide gels in both the non-reducing and reducing [with β-mercaptoethanol (Bio-Rad Laboratories, Hercules, California, USA)] condition. Electrophoresis was conducted at 200V for 45–60 min using the Mini-Protean® System (Bio-Rad, Hercules, California, USA). The proteins were fixed and then visualized with Coomassie Brilliant Blue (Bio-Rad Laboratories, Hercules, California, USA), photographed and dried. The relative component proteins were compared with each other and against the molecular weight marker (Precision Plus Protein® Standard, Bio-Rad Laboratories, Hercules, California, USA) bracketing the samples.

Development of fern recipes of the ten species of edible ferns – For this part of the study, a fern gourmet contest was held in the Center for Biodiversity Research and Extension in Mindanao (CEBREM) in CMU, Musuan, Bukidnon. The fern gourmet contest was open to the public as announcements were made in the university and neighboring places including in restaurants and cafeterias. We supplied all cooking utensils and stove, and most importantly all the basic food materials and special requests as deemed justified. The ten species of ferns for this contest were obtained from the Pteridogarden, Mt. Musuan Botanical Garden.

On the day of the contest there was an orientation to all the participants in terms of rules and guidelines for the contest. The fern gourmets prepared for the contest were evaluated by a Sensory Panel composed of faculty, staff, and food enthusiasts who are well-trained in evaluating quality of food, way of cooking, taste and component variations of food. A set of criteria was used as guideline by the Sensory Panel, viz., 30% Originality, 30% Palatability, 20% Workmanship and 20% Presentation.

Information dissemination to the public of the health and wellness benefits of edible ferns – In order to disseminate the results demonstrating the re-introduction of edible ferns, brochures were prepared on the planting and propagation of the ten species of edible ferns. A seminar-field visit was held and participated by farmers and local government units (LGU). Cooking demonstration on edible ferns was done by the winners of the gourmet contest.

Results:-

Preliminary test for antioxidant by the FeCl₃ test – Results from this preliminary screening test indicated that all the ten species of Mindanao indigenous edible ferns contain antioxidants as shown by brownish green to intense dark purple/blue coloration with precipitate on FeCl₃ test (Table 1).

Free Radical Scavenging Activity of the Methanol Extract – Results of the DPPH assay showed highest percent activity at 76.0%± 7.0% for *D. esculentum*, 53.5±2.5% for *M. crenata*, 52.5±0.5% for *S. glauca*, 48.0±0.0% for *A. nidus*, 45.0±5.0% for *A. aureum*, 42.5±4.5% for *C. thalictroides*, 39.0±8.0% for *P. ensiformis*, 38.5±4.5% for *P. aquilinum*, 34.5±5.5% for *A. palmiformis* and 32.5±1.5% for *S. palustris*. The results of Analysis of Variance (ANOVA) showed that they have significantly different free radical scavenging activity ($F_{9,50}=40.06$, P less than 0.05).

The corresponding antioxidant value for each fern species reported as per gram dry weight and per gram wet weight is presented in Fig. 3. Data from antioxidant as μmol ascorbic acid per gram wet weight (as vegetables) showed that *M. crenata* has the highest value of 152±7 μmol ascorbic acid per gram wet weight. The results of ANOVA of free radical scavenging activity of the ten species of ferns per wet weight basis are significantly different from each other ($F_{9,50}=126$, P less than 0.05).

Phytochemical Screening – As shown in Table 2, all the ten species contain alkaloids, saponins, phenolic compounds, flavonoids and only 4 species with terpenes. Terpenes are confirmed positive in *P. ensiformis*, *D. esculentum*, *P. aquilinum* and *A. nidus*.

Protein Content – Data from the Bradford test of the ten indigenous edible ferns show efficient extraction method resulting in concentrations ranging from 0.58 to 0.67mg/mL. The values as mg protein/g wet weight were calculated and compared to indicate the protein content of the indigenous edible ferns when consumed as vegetables. The values range from 0.1 to 4.4mg protein/g wet weight with *M. crenata* having the highest protein content as vegetables (Fig. 4). The results of ANOVA of protein content of the ten species of ferns per wet weight basis are significantly different from each other ($F_{9,10}=28.00$, P less than 0.05).

Relative Component Proteins by SDS-PAGE – At the beginning of the study, we were heating the mixture of Laemmli sample load buffer and the fern frond samples. By this method, we observed inconsistent extraction of the proteins with unresolved bands observed in the electrophoresis gels. Later we decided not to heat the mixture but instead leaving it at room temperature for 30 min. It was only in this method that we obtained consistent extraction. Heating can affect the integrity of some proteins because of denaturation and aggregation brought about by non-specific interactions at high temperature. In general, at lower temperature the integrity of proteins is maintained.

Results in Fig. 5 indicate that no two fern species are the same. *M. crenata*, *A. aureum*, *S. palustris*, *D. esculentum*, and *P. aquilinum* have proteins with molecular weights ranging from approximately 12-250kDa whereas *P. ensiformis* is approximately 18-250kDa and *S. glauca* ranged from 25-90kDa. *C. thalictroides* has molecular weight ranging from 15-250kDa. *A. palmiformis* has molecular weight ranging from 12-60kDa whereas *A. nidus* is the fern species with the lowest molecular weight protein (molecular weight ranging from 10-250kDa). All these fern species contain proteins with molecular weight at approximately 25kDa and 60kDa.

In this study, we can also deduce from the electrophoretic profiles that there are some species of these ferns with more protein bands in the reducing condition than in the non-reducing condition indicating that these ferns have proteins containing subunits connected by disulfide bond. These are *M. crenata* (additional bands at approximately 20 and 30 kDa), *P. ensiformis* (additional band at approximately 18 kDa), *A. aureum* (additional bands at approximately 16 and 20 kDa), *S. palustris* (additional band at approximately 16 kDa), *P. aquilinum* (additional bands at approximately 15 and 90 kDa), *A. nidus* (additional at approximately 60 kDa) and *C. thalictroides* (additional band at approximately 60 kDa). *D. esculentum*, *A. palmiformis* and *S. glauca* have the same component proteins for both the non-reducing and reducing condition indicating that their component proteins are composed of one unit.

Initial step to re-introduce 10 species of ferns as food – Participants in the Fern Gourmet contest included students, staff, caterers, and food enthusiasts. A total of 26 dishes with at least 2 dishes per fern species were

prepared. Figure 6 shows the winning dishes for the 10 edible fern species. Propagation and harvesting protocols were disseminated in the form of brochures as well as in fern gourmet recipe book (Fig. 7) during the seminar.

Discussion:-

Antioxidant Activity and Phytochemical Screening – Bioactivity and phytochemical investigation of edible ferns is scarce in the literature except for one or two of these ten species of ferns. In terms of use of fern fronds for food, the young sterile tender fronds are more acceptable, which has been the practice for leafy vegetables in many parts of the Philippines as well as in other countries (de Winter and Amoroso, 2003). In the present study, the preliminary antioxidant screening test with $FeCl_3$ was confirmed in the phytochemical screening test by TLC and the quantitative DPPH test. Results from phytochemical screening gave definite positive results for both the phenolics and flavonoids indicating high potential for antioxidant activity, which is demonstrated in the DPPH assay results in all the ten edible ferns included in this study. Notable is *M. crenata* with the highest activity as vegetable (per wet weight).

D. esculentum is the fern species that is readily available and widely used as human food throughout the Philippines. The young sterile tender fronds are eaten as salad with various dressing, cooked as leafy vegetable or as ingredient of stew or pickled (Zamora and Co, 1986). Moreover, this species is easy to propagate and grows throughout the year. It produces root buds which in turn become new plants in a short period ensuring continuous supply. *M. crenata*, on the other hand, is widespread in Mindanao but not utilized as food plant. However, this fern is used as vegetable in soup dishes only in the province of Iloilo (Visayan region). Both *D. esculentum* and *M. crenata* are obtained from the wild when utilized as vegetable and have not been domesticated in the Philippines. The other eight species of edible ferns included in this study are not yet utilized as part of the Filipino diet. These species contain comparable antioxidant activity with that of *D. esculentum* and *M. crenata* and therefore can also be promoted as functional food to combat diseases due to lifestyle.

Results in the present study on the presence of antioxidants in fern samples are supported by Carcia et al., 2006; Chen et al., 2007; Ding et al., 2008; Hadifh et al., 2009; Shin and Lee, 2010. Korean researchers also conducted studies on the antioxidant activity of 37 fern and fern allies species (Lee and Shin, 2010). Many of these ferns, i.e., Dryopteridaceae, Osmundaceae, Woodsiaceae, showed potential antioxidant activities including *Polystichum lepidocoulon* and *Polystichum polyblepharum* which contains 13% of total polyphenols.

Many secondary metabolites or phytochemicals are discovered in ferns but not in other plants (Zhao et al., 2007; and Shinozaki et al., 2008). These phenomena are theorized to be caused by the adaptation undergone by these species from different stresses and natural factors dated back from Paleozoic times up to the present (Wallace et al., 1991).

Protein Content – Proteins, like any other molecules, are very essential since they can store energy or be a catalyst in biochemical reactions. For the ten edible ferns in the present study all contain quantifiable amounts of proteins and reported as protein per wet weight to indicate their benefit as vegetables.

Proteins from different plants including ferns had been determined as reported in the literature but most of them are still using the proximate analysis reported as crude protein content using total inorganic nitrogen determination by the Kjeldahl method. The use of Bradford assay on plant proteins over the Kjeldahl method and BSA reference standard in lieu of the expensive dominant form of foliar protein, an enzyme, ribulose 1,5-diphosphate carboxylase-oxygenase (RUDP) was extensively studied on plant proteins by Jones et al. (1989). The data indicated no significant difference in dye response between BSA and RUDP. The values obtained from Kjeldahl method cannot be compared with the direct Bradford assay for proteins that uses a reference standard.

The quantifiable amount of proteins in these ten edible ferns could help combat the protein deficiency in the Philippines. Finding an alternative source of protein is a strategy towards achieving food sufficiency, security and dietary balance. The incorporation of plant proteins for food products has been the subject of many investigations (Young and Pellet, 1994; Zara et al., 2013; Essuman et al., 2014). So, determination of protein concentration in plants is important. However, there are only limited studies regarding the protein content of ferns except in defatted fern fronds of *Nephrolepis bisserata* and *Arthropteris orientalis* (Essuman et al., 2014).

Another test for the presence of proteins in the ten edible ferns in this study is the SDS-PAGE stained with Coomassie. We are the first one to report the relative component proteins of edible ferns by SDS-PAGE. In this

study, results from SDS-PAGE indicate that no two edible ferns included in the present study are the same and that the component proteins of some of these fern species are composed of more than one unit linked by disulfide bond. Our data show that the SDS-PAGE technique can be an important tool to determine the protein electrophoretic profile of ferns for determining the identity of species of ferns in addition to morphological and taxonomic techniques. SDS-PAGE has also been reported as a tool to explore evolutionary relationships on some seed storage proteins (Turi et al., 2010; Zada et al., 2013; Essuman et al., 2014).

Although plant proteins are known to have low proportion of sulfur-containing amino acids methionine and cysteine compared to animal proteins, it appears that in seven of the fern species in this study, cysteine definitely formed inter- disulfide bonds leading to their reduction in the presence of the reducing agent β -mercaptoethanol.

Food preparation techniques and dissemination to the public – Based on the results on the fern gourmet contest held, we then established protocols on handling the ferns as vegetables and indicated their characteristics when used as food in 26 dishes (Amoroso et al., 2014). The seminar-field visit, the fern recipe book and information, education, and communication (IEC) materials on fern propagation and cultivation were used to disseminate the information we gathered from the study. The edible ferns and fern recipe book presents information on antioxidants and protein content of each ten edible species of ferns. The book contains recipes that not only prove the worth of these usually neglected edible ferns through the delicious and sumptuous dishes, but also showcase their healthful benefits. The book serves as an important tool in building public awareness on the importance of edible ferns and its many innovative uses in the culinary world. The IEC materials emphasize that these edible ferns are low cost and requires no chemicals in their cultivation. Dissemination of the results from this study and the publication materials will encourage the Filipino people in eating varied vegetables to include these under-utilized, cultivated, edible ferns and adhere to home cooking than depending on fast foods.

For convincing the public of the benefits of certain vegetables or food that are not popular, scientific data is a requirement. The way to do this is to present evidences of what are present in the materials beneficial to health, their palatability, and importantly their availability. In the present study, we demonstrated the radical scavenging activity of these ten indigenous edible species of ferns as well as their protein content and preliminary assessment of the component proteins in SDS-PAGE. Our results show that the methanol extracts of the young sterile tender fronds of these ten species of ferns have quantifiable radical scavenging activity as well as proteins. The relative component proteins of these ferns show unique profiles. No two fern species have the same profile.

The palatable *M. crenata* grows in rice paddies and moist soils produce young sterile tender fronds that can be harvested once a week. This species has the highest radical scavenging activity as well as protein content as a vegetable. On the other hand, *D. esculentum* is so popular because of its abundance with a biomass of young fronds that can be harvested almost every other day. It has radical scavenging activity comparable to that of ascorbic acid. *D. esculentum* is more tender in texture, palatable and highly acceptable that multiple food recipes can be prepared from it as salad, as vegetable in soup, in special treats, etc.

S. palustris, *P. ensiformis*, *A. aureum*, *A. nidus* and *A. palmiformis* are also available all throughout the year and can easily be propagated. These five fern species easily adapt to the environment once their roots are established on the soil. Aside from their high antioxidant activity and protein content as vegetables, they are also palatable. Recipes out of these ferns can easily be prepared.

Among the ten edible fern species, *S. glauca*, *C. thalictroides* and *P. aquilinum* have comparatively low antioxidants and protein content. Based on our study regarding propagation of the ten species of ferns, these three species are difficult to domesticate in the garden and therefore are less available throughout the year.

Future Work – Large-scale farming of the edible ferns will be done through asexual and *in vitro* propagation including the development of alternative protocols that are less expensive on the part of the farmers. Cost and return analysis for the recommended edible fern species will be made to determine their feasibility as source of income. Product development, technology commercialization and packaging of products made of these edible fern species will be conducted.

Table 1:- Observation on the appearance of indigenous edible fern extracts upon addition of 10% Ferric Chloride

Scientific Name	Visible observation (color change)	Remarks
1. <i>Marsilea crenata</i>	Orange to Greenish brown with precipitate	+
2. <i>Pteris ensiformis</i>	Orange to Reddish brown	+
3. <i>Acrostichum aureum</i>	Light yellow to Brownish yellow	+
4. <i>Stenochlaena palustris</i>	Red to Purple with precipitate	+
5. <i>Diplazium esculentum</i>	Light orange to Greenish brown with precipitate	+
6. <i>Angiopteris palmiformis</i>	Colorless to Light Orange	+
7. <i>Pteridium aquilinum</i>	Orange to Light brown with precipitate	+
8. <i>Sphaeropteris glauca</i>	Orange to Purplish green/blue with precipitate	+
9. <i>Asplenium nidus</i>	Light orange to Light brown with precipitate	+
10. <i>Ceratopteris thalictroides</i>	Orange to Dark brown with precipitate	+

+ Indicates presence of antioxidants

Table 2:- Phytochemical screening using Thin Layer Chromatography

Species	Dragendorff's Test (Alkaloids)		Liebermann-Burchard Test (Saponins)		Potassium ferric cyanide-ferric chloride Test (Phenolics)		Antimony (III) chloride Test (Terpenes)		Vanillin/Sulfuric Acid Test (Flavonoids)	
	Color of spot	Remarks	Color of spot	Remarks	Color of spot	Remarks	Color of spot	Remarks	Color of Spot	Remarks
<i>M. crenata</i>	Brown Orange	+	Pink	+	Blue	+	Faint red spot	+/-	Red	+
<i>P. ensiformis</i>	Brown Orange	+	Pink	+	Blue	+	Red	+	Red	+
<i>A. aureum</i>	Brown orange	+	Pink	+	Blue	+	Faint red spot	+/-	Red	+
<i>S. palustris</i>	Brown orange	+	Pink	+	Blue	+	Faint red spot	+/-	Red	+
<i>D. esculentum</i>	Brown orange	+	Pink	+	Blue	+	Red	+	Red	+
<i>A. palmiformis</i>	Brown orange	+/-	Pink	+	Blue	+	Faint red spot	+/-	Red	+
<i>P. aquilinum</i>	Brown orange	+	Pink	+	Blue	+	Red	+	Red	+
<i>S. glauca</i>	Brown orange	+	Pink	+	Blue	+	Faint red spot	+/-	Red	+
<i>A. nidus</i>	Brown orange	+	Pink	+	Blue	+	Red	+	Red	+
<i>C. thalictroides</i>	Brown orange	+	Pink	+	Blue	+	Faint red spot	+/-	Red	+

|+| - intense bands (definitely positive)

|+/-| - faint bands

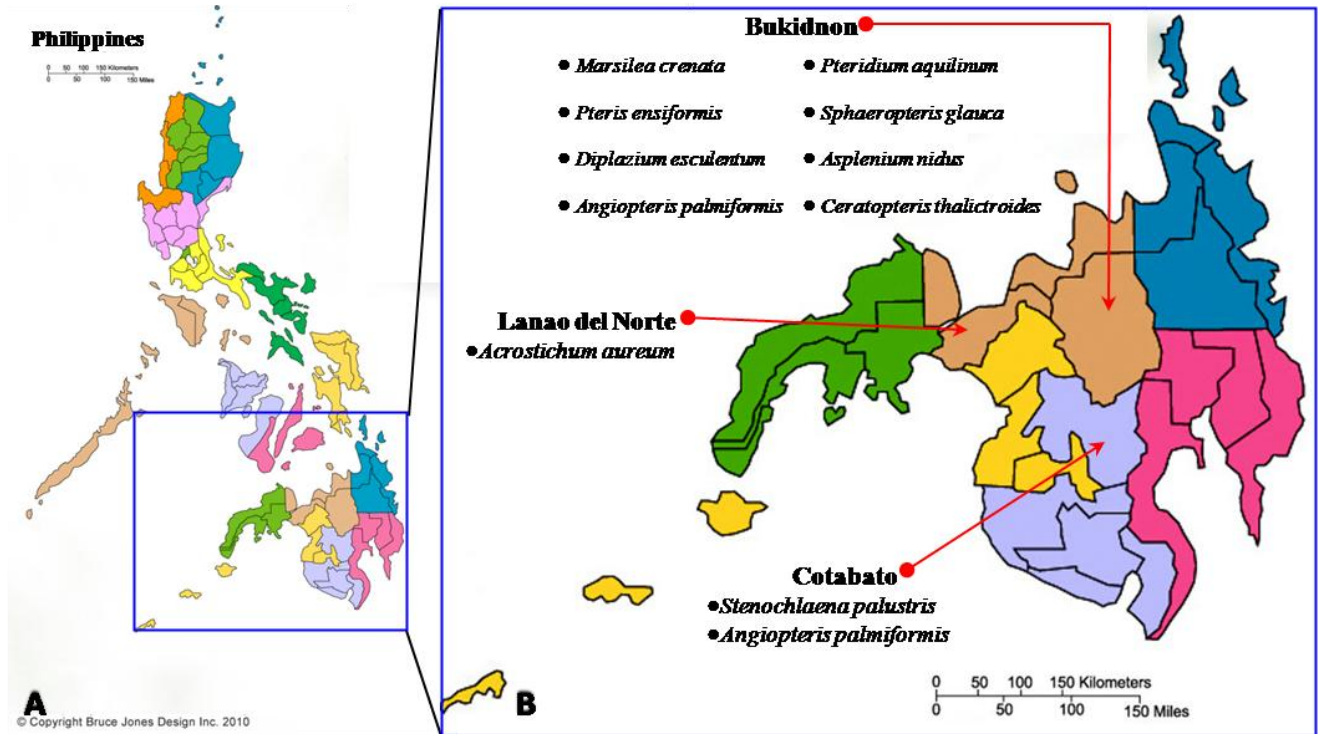


Fig 1:- A. Map of the Philippines B. Enlarged map of the island of Mindanao indicating the distribution of 10 species of edible ferns.



Fig 2:- Habit of the 10 edible species of ferns.

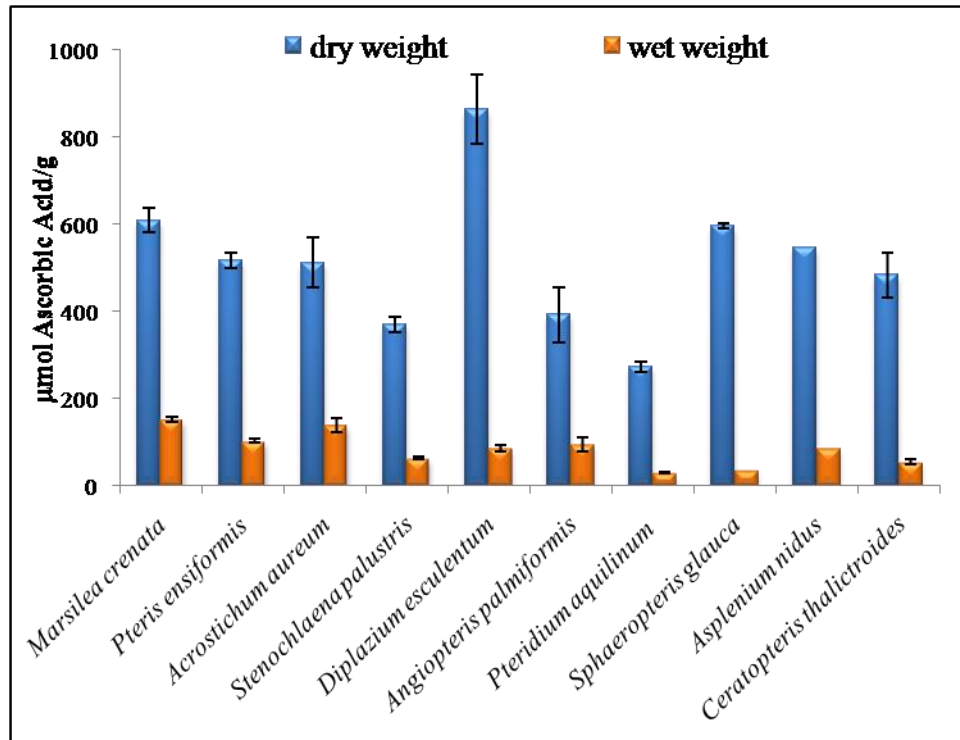


Fig3:- Comparison of antioxidant activity of the Mindanao ferns per sample weight

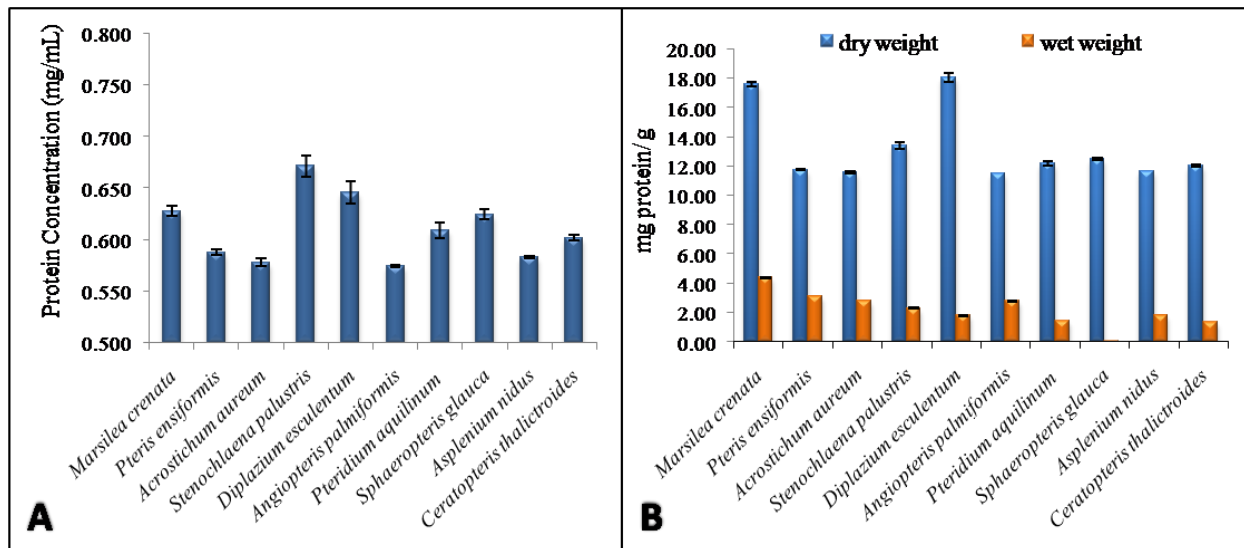
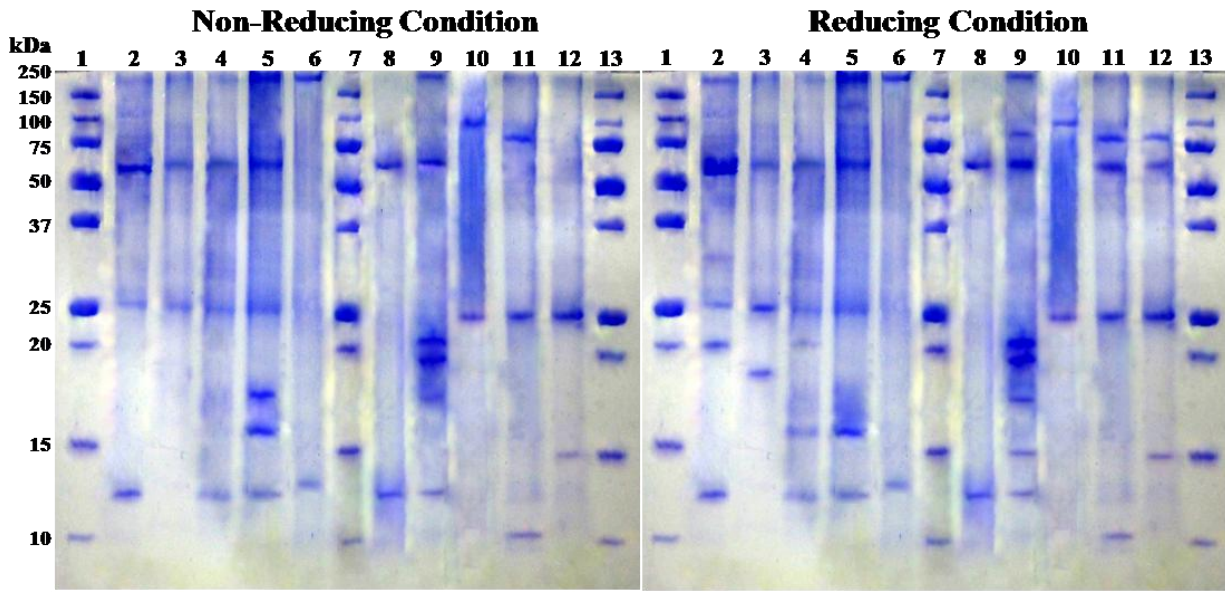


Fig 4:- A. Comparison of total protein concentration in Mindanao edible ferns determined by the Bradford method; B. Comparison of total protein content in Mindanao edible ferns relative to sample weight



Well No.

1. Precision Plus Mol. Wt. Marker	6. <i>Diplazium esculentum</i>	11. <i>Asplenium nidus</i>
2. <i>Marsilea crenata</i>	7. Precision Plus Mol. Wt. Marker	12. <i>Ceratopteris thalictroides</i>
3. <i>Pteris ensiformis</i>	8. <i>Angiopteris palmiformis</i>	13. Precision Plus Mol. Wt. Marker
4. <i>Acrostichum aureum</i>	9. <i>Pteridium aquilinum</i>	
5. <i>Stenochlaena palustris</i>	10. <i>Sphaeropteris glauca</i>	

Fig5:- SDS-PAGE profile of the ten edible ferns



Fig6:- Winning fern gourmets evaluated by a sensory panel

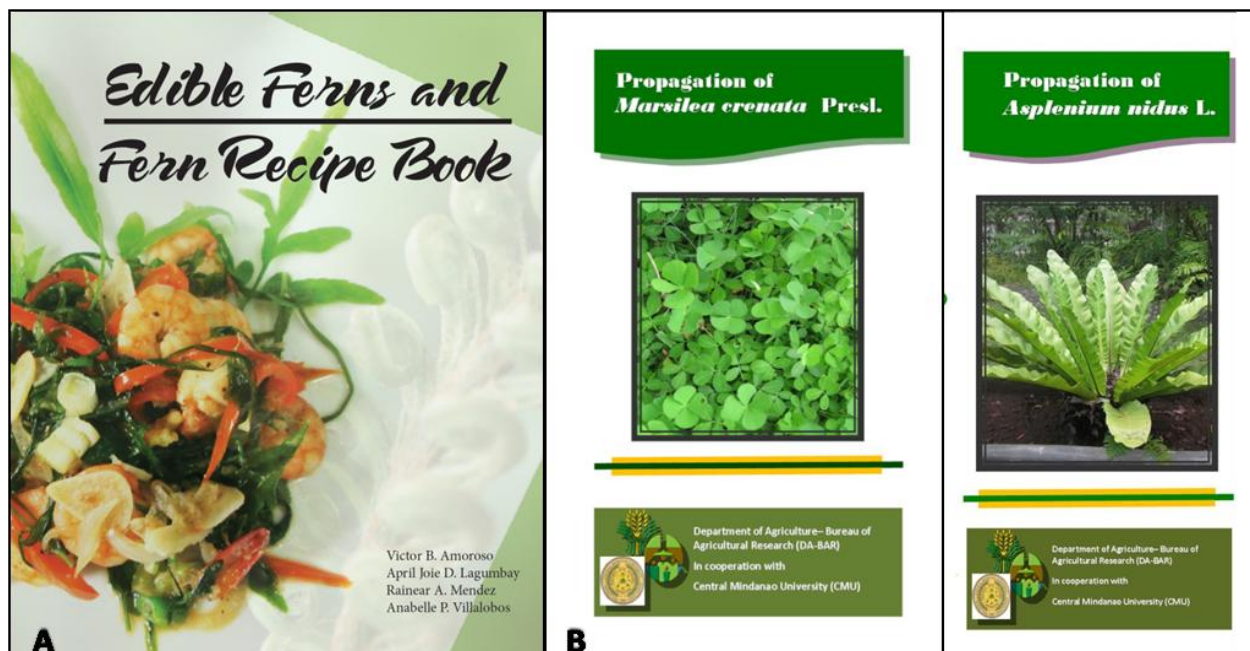


Fig7:- A. Edible Ferns and Fern Recipe Book and; B. Selected Protocols for Planting, Propagation and Harvesting of the Ten Edible Species of Ferns

Acknowledgment:-

The authors thank the Department of Agriculture-Bureau of Agricultural Research (DA-BAR), the Philippine Council for Agriculture, Aquatic & Natural Resources Research & Development (PCAARRD), and the Philippine Center on Higher Education (CHED) for funding this study, the administration of Central Mindanao University, the Department of Chemistry of the University of Cincinnati, and Johnson & Johnson Pharmaceutical R&D & Ms. Joie D. Lagumbay for the laboratory activities.

Literature Cited:-

1. Afriastini, J.J. (2003): *Marsilea crenata* C. Presl. In: de Winter, WP and Amoroso, VB (eds): Plant Resources of South-East Asia No 15(2). Cryptograms: Ferns and fern allies. Backhuys Publishers, Leiden, The Netherlands. pp. 133-135.
2. Amoroso, V.B. (1990): Ten Edible Ferns of Mindanao. Philippine Journal of Science. 119 (4): 295-313.
3. Amoroso, V.B. (2013): Philippine Medicinal Ferns and Lycopods. Central Mindanao University, Musuan, Bukidnon.
4. Amoroso, V.B., Obsioma, L.D., Arlalejo, J.B., Aspiras, R.A., Capili, D.P., Polizon, J.J.A. and Sumile, B. E. (2009): Inventory and conservation of endangered, endemic and economically important flora of Hamiguitan Range, Southern Philippines. Blumea 54, 71-76.
5. Amoroso, V.B., Laraga, S.H. and Calzada, B.V.(2011): Diversity and assessment of plants in Mt. Kitanglad Range Natural Park, Bukidnon, Southern Philippines. Garden's Bulletin Singapore 63 (1 & 2): 219-236.
6. Amoroso, V.B., Lagumbay, A.J.D., Mendez, R.A. and Villalobos, A.P. (2014): Edible Ferns and Fern Recipe Book. Bureau of Agricultural Research, Department of Agriculture, Quezon City, Philippines.
7. Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. May 7;72:248-54.
8. Benjamin, A. and Manickam, V.S. (2007): Medicinal pteridophytes from the Western Ghats. Indian J. Trad. Knowl. 6:611-618.
9. Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004): Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74:2157-2184.
10. Carcia, F., Pivel, J.P., Guerrero, A., Brieva, A., Martinez-Alvarez, M.P., Caamano-Somoza, M. and Conzlez, S. (2006): Phenolic components and anti-oxidant activity of Fernblock, an aqueous extract of the aerial parts of the fern *Polypodium leucotomos*. Methods Find Exp. Clin. Pharmacol. 28: 157-160.

11. Chai, T.T., Panirchellvum, E., Ong, H.C. and Wong, F.C. (2012): Phenolic contents and antioxidant properties of *Stenochlaena palustris*, an edible medicinal fern. *Botanical Studies* 53:439-446.
12. Chen, Y.H., Chang, F.R., Lin, Y.J., Wang, L., Chen, J.F., Wu, Y.C. and Wu, M.J. (2007): Identification of phenolic antioxidants from Sword Brake fern (*Pteris ensiformis* Burm.). *Food Chem.* 105:48-56.
13. Dai, J. and Mumper, R.J. (2010): Plant Phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15: 7313-7352.
14. Ding, Z.T., Fang, Y.S., Tai, Z.G., Yang, M.H., Xu, Y.Q., Li, F. and Cao, Q.E. (2008): Phenolic content and radical scavenging capacity of 31 species of ferns. *Fitoterapia* 79: 581-583.
15. de Winter, W.P. and Amoroso, V.B. (2003): *Plant Resources of South-East Asia No 15(2). Cryptograms: Ferns and fern allies.* Backhuys Publishers, Leiden, The Netherlands. 268 pp.
16. Essuman, E.K., Ankar-Brewoo, G.M., Ofosu, I.W. and Barimah, J. (2014): Optimization Of Protein Extraction From Fern Frond Using Response Surface Methodology (RSM). *Ife Journal of Science* 16(1).
17. Gil, M.I., Tomas-Barberan, F.A., Hess-Pierce, B. and Kader, A.A. (2002): Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry* 50, 4976-4982.
18. Hafidh, R.R., Abdulamir, A.S., Abu Bakar, F., Abas, F., Jahanshiri, F. and Sekawi, Z. (2009): Antioxidant Research in Asia in the Period from 2000-2008. *Am. J. Pharmacol. And Toxicol.* 4(3):48-66.
19. Ho, R., Teai, T., Bianchini, J.-P., Lafont, R. and Raharivelomanana, P. (2010): Ferns: From traditional uses to pharmaceutical development, chemical identification of active principles. In: Fernandez H, Revilla MA, Kumar A (eds.), *Working with ferns: Issues and applications.* Springer, New York, pp. 321-346.
20. Huang, D., Our, B. and Prior, R.L. (2005): The Chemistry Behind the Antioxidant Capacity Assays. *J. Agric. Food Chem.* 53, 1841-1856.
21. Jin, H.M., Kang, S.J. and Lee, S.H. (2005): Cosmetic composition for scrubbing. *PCT Int. Appl., WO/2005/082328, KO Appl. 1020050016923.*
22. Jones, C.G., Hare, J.D. and Compton, S.J. (1989): Measuring plant protein with the Bradford Assay. *Journal of Chemical Ecology*, 15(3).
23. Katsube, T., Imawaka, N., Kawano, Y., Yamazaki, Y., Shiwaku, K. and Yamane, Y. (2006): Antioxidant flavonol glycosides in mulberry (*Morus alba* L.) leaves isolated based on LDL antioxidant activity. *Food Chem.* 97:25-31.
24. Kim, H. J., Lim, H.W., Choi, S.W. and Yoon, C.S. (2006): Antimicrobial effect of ethanol extract of *Dryopteris crassirhizoma* Nakai on *Propionibacterium acnes*. *J. Soc. Cosmet. Sci. Korea* 32:201-208.
25. Lee, C.H. and Shin, S.L. (2010): Functional activities of ferns for human health. In: Fernández H, Revilla MA, Kumar A (eds.), *Working with ferns: Issues and applications.* Springer, New York, pp. 347-359.
26. Marañon, J. (1935): Nutritive Mineral Value of Philippine Food Plants (Calcium, Phosphorus, and Iron Content). *The Philippine Journal of Science* 58:317-358.
27. Miller, H.E., Rigelhof, F., Marquat, L., Prakash, A. and Kanter, M. (2000): Anti-oxidant content of whole grain breakfast cereals, fruits and vegetables. *J. Am. Coll Nutr.* 19(3 Suppl):312S-319S.
28. Mosquera, O.M., Correa, Y.M., Buitrago, D.C. Niño, J. (2007): Antioxidant Activity of Twenty Five Plants from Colombian Biodiversity. *Mem Inst Oswaldo Cruz, Rio de Janeiro*, 102 (5), 631-634.
29. Ou, B., Hampsch-Woodill, M. and Prior, R.L. (2001): Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem* 49, 4619-4626.
30. Park, H.A., Kweon, M.H., Han, H.M., Sung, H.C. and Yang, H.C. (1998): Effects of the glycoprotein isolated from *Pteridium aquilinum* on the immune function of Mice. *Korean J. Food Sci. Technol.* 30:976-982.
31. Parvez, S., Kang, M., Chung, H.S., Cho, C., Hong, M.C., Shin, M.K. and Bae, H. (2006): Survey and mechanism of skin depigmenting and lightening agent. *Phytother. Res.* 20:921-934.
32. Prakash, A, Rigelhof, F. and Miller, H.E. (2011): Anti-Oxidant Activity. *Medallion Laboratories Analytical Progress*, J. de Vries, ed.
33. Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B. and Jacob, R. (2003): Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *J. Agric. Food Chem* 51, 3273-3279.
34. Procházková, D., Boušová, I. and Wilhelmová, N. (2011): Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* 82: 513-523.
35. Pyrzynska, K., and Pękal, A. (2013): Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Anal. Methods*, 2013, 5, 4288.

36. Rathore, G.S., Suthar, M., Pareek, A. and Gupta, R.N. (2011): Nutritional antioxidants: A battle for better health. *J. Nat. Pharm.* 2:2-14.
37. Shin, S.L. and Lee, C.H. (2010): Antioxidant effects of the methanol extracts obtained from aerial part and rhizomes of ferns native to Korea. *Korean J. Plant Res.* 23:38–46.
38. Shinozaki, J., Shibuya, M., Masuda, K. and Ebizuka, Y.(2008): Squalenecyclase and oxidosqualenecyclase from a fern. *FEBS Lett.* 582:310-318.
39. Simopoulos A P. (2004): Omega-3 fatty acids and antioxidants in edible wild plants. *Biol. Res.* 37: 263-277.
40. Smith, R.A., Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H. and Wolf, P.G. (2006): A classification for extant ferns. *TAXON* 55 (3), 705-731.
41. Swain, T. (1977): Secondary compounds as protective agents. *Ann. Rev. Plant Physiol.* 28:479-501.
42. Tanaka, S., Sato, T., Akimoto, N., Yano, M. and Ito, A. (2004): Prevention of UVB – induced photoinflammation and photoaging by a polymethoxy flavonoid, nobilerin, in human keratinocytes in vivo and in vitro. *Biochem. Pharmacol.* 68:433–439.
43. Turi, N., Farhatullah, Rabbani, M.A., Khan, N.U. Akmal, M., Pervaiz, Z.H. and Aslam, M.U. (2010): Study of total seed storage protein in indigenous Brassica species based on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). *Af.J. Biotech.* 9(45), 7595-7602.
44. Usman H, Abdulrahman, F.I. and Usman, A. (2009): Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficusthonningii* (Moraceae). *Afr J Tradit Complement Altern Med.* 6(3):289–295.
45. Wallace, R.A., Sander, G.P. and Ferl, R.J. (1991): *Biology: the science of Life*. New York. Harper Collins, pp 547-555.
46. Young, V.R. and Pellett, P.L. (1994). Plant protein in relation to human protein and amino acid nutrition. *Am. J. Clin. Nutr.* 59(suppl):1203S-12S.
47. Zada, M., Shinwari, Z.K., Zakir, N. and Rabbani, A. (2013): Study of Total Storage Proteins in Ethiopian Mustard (*Brassica caritana* A. Braund) Germplasm. *Pak. J. Bot.* 45(2)443-338.
48. Zhao, Z., Jin, J., Ruan, J., Zhu, C., Fang, W. and Cai, Y. (2007): Antioxidant flavonoid glycosides from aerial parts of the fern *Abacopteris penangiana*. *J. Nat. Prod.* 70:1683-1.