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Preparation of Natural Antioxidant Health Supplements from Philippine-Grown Medicinal Plants

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Abstract

Natural antioxidants are molecules that prevent cell damage against free radicals and are significant for maintaining optimum health in both animals and human. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress which contributes to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis and neurodegeneration in motor neuron diseases. Due to the importance of natural antioxidants in the prevention of these diseases, this study was therefore undertaken to extract, characterize and evaluate the antioxidant activity of some Philippine-grown medicinal plants for the development of natural antioxidant dietary supplements. The collected plant materials namely *Fragaria vesca* (strawberry), *Solanum melongena* (eggplant), *Nephelium lappaceum* (rambutan), *Mangifera indica* (mango), *Antidesma bunius* (bignay), *Basella rubra* (alugbati), *Garcinia mangostana* (mangosteen), *Syzygium cumini* (duhat), *Dioscorea alata* (ube), *Citrus grandis* (suha), *Annona muricata* (guyabano) and *Curcuma longa* (turmeric) were extracted using 95% EtOH. The total phenolic content of the plant extracts was tested by Folin-Ciocalteu method. Flavonoid content of the plant was determined by qualitative phytochemical analysis. The study also prepared a natural-based antioxidant dietary supplement product in the form of capsule and chewable tablet that contains a combination of two (2) to three (3) plant materials that exhibited the most promising antioxidant activity.

Results suggest that *N. lappaceum* peels exhibited the highest antioxidant activity with 40.70% total phenolics expressed as gallic acid followed by *G. mangostana* pericarp at 29.00% and *S. cumini* fruit at 14.30%. All the plant samples indicated the presence of flavonoids. An antioxidant dietary supplements in capsule and in chewable tablet were developed using a combination of two (2) to three (3) plant extracts. The formulated products exhibited very promising antioxidant activities.

The antioxidant activities exhibited by some Philippine-grown medicinal plants lead to the preparation of a more sustainable and cost effective natural antioxidant dietary supplements.

Keywords: Antioxidant; Bate-Smith and Metcalf method; DPPH assay; Folin-Ciocalteu method; Medicinal plants; Phytochemical analysis; Wilstatter "Cyanidin" test

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Introduction

When excessive quantities of reactive oxygen and/or nitrogen species overshadow the endogenous antioxidative capability of the cells, an imbalance state called oxidative stress occurs. This state may be harmful due to the free radicals that stimulates the oxidation of macromolecules such as proteins, enzymes, lipids and DNA which leads to several serious diseases [1,2]. However, the destructive action of the free radicals can be blocked by antioxidant substances which scavenge the free radicals and detoxify the cells. Antioxidants are defined as compounds that can prevent or delay the oxidation of oxidizable products in the body by scavenging free radicals and reducing oxidative stress. It is said that the body has antioxidant defense systems to fight the harmful effects caused by free radicals but due to the malfunction of the antioxidant defense system, it needs to be supplemented from outside sources. Antioxidants can be found naturally in plants and food. Eating plenty of antioxidants is one of the best ways to prevent oxidative stress caused by free radicals.

The present study was designed to investigate the antioxidant capacity of some Philippine-grown medicinal plants through Folin-Ciocalteu method and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay and to prepare natural antioxidant dietary supplements from the combination of the tested plants.

Materials and Methods

Preparation of Plant Extract

Plant materials such as leaves of *Basella rubra*, *Syzygium cumini*, and *Annona muricata*; fruits and leaves of *Antidesma bunius*; fruits of *Solanum melongena* and *Fragaria vesca*; peels/pericarp of *Garcinia mangostana* and *Mangifera indica*; rhizomes of *Curcuma longa*; peels of *Nephelium lappaceum* and *Citrus grandis*; and *Dioscorea alata* were used in this study. These plant materials were collected from different regions/ places in the Philippines. The plant materials were labelled and authenticated. The plant materials were standardized following the general protocol (Manual on the ICS-UNIDO Training on Quality Control of Medicinal and Aromatic Plants and Their Products, 1998).

Phytochemical Analysis and Antioxidant Assays

The crude plant extracts were evaluated to determine the presence of flavonoids and leucoanthocyanins following the Wilstatter “Cyanidin” test and Bate-Smith and Metcalf method (Guevara, 2005). Meanwhile, the total phenolic content of the plant extracts were determined according to Folic-Ciocalteu method (Singleton and Rossi, 1965).

Natural Antioxidant Health Supplement Development

For product preparation, the ground plant material was further sieved and weighed. About 250-300 mg powder of a combination of

two (2) to three (3) plant materials with high antioxidant activity were weighed. Three (3) formulations of antioxidant capsule and two (2) formulations of chewable tablet were prepared. Formula 1 capsule is comprised of 2 active components of *G. mangostana* and *C. longa* (1:1). Formula 2 capsule is comprised of *A. muricata* and *G. mangostana* (1:1). Formula 3 capsule was formulated with the combination of *C. longa* and *A. muricata* (1:1). These 3 formulations were tested for % total phenolic content expressed as gallic acid. On the other hand, Formula 4 chewable tablet comprised of three active components of *G. mangostana*, *N. lappaceum* and *F. vesca* extract (2:3:1). Formula 5 of antioxidant chewable tablet had active components of *N. lappaceum*, *A. bunius* and *G. mangostana* extract (3:1:2) with the ratio of, respectively. These two (2) formulations were tested to determine their % total phenolic content, and free radical scavenging activities by the DPPH assay.

Table 1.1: Summary of Formula 1-3.

	Formula 1	Formula 2	Formula 3
Type	capsule	capsule	capsule
Components	<i>G. mangostana</i> ; <i>C. longa</i> (1:1)	<i>A. muricata</i> ; <i>G. mangostana</i> (1:1)	<i>C. longa</i> ; <i>A. muricata</i> (1:1)
Plant part	Pericarp; rhizomes	Leaves; pericarp	Rhizomes; leaves

Table 1.2: Summary of Formula 4 and 5.

	Formula 4	Formula 5
Type	chewable tablet	chewable tablet
Components	<i>G. mangostana</i> ; <i>N. lappaceum</i> ; <i>F. vesca</i> (2:3:1)	<i>G. mangostana</i> ; <i>N. lappaceum</i> ; <i>A. bunius</i> (3:1:2)
Plant part	Pericarp; peels; fruit	Pericarp; peels; fruits and leaves

Results

The results of the phytochemical analysis of the plant extracts were shown in Table 1. All the twelve (12) tested plant extracts indicated the presence of flavonoids.

Table 2: Results of the qualitative phytochemical analysis of the plant extracts.

Plant Extract	Wilstatter “Cyanidin” Test	Bate-Smith and Metcalf Method
<i>A. bunius</i> fruits and leaves	positive	positive
<i>A. muricata</i> leaves	positive	positive
<i>B. rubra</i> leaves	positive	positive
<i>C. grandis</i> peels	positive	positive
<i>C. longa</i> rhizomes	positive	positive
<i>D. alata</i> tubers	positive	positive
<i>G. mangostana</i> pericarp	positive	positive
<i>F. vesca</i> fruit	positive	positive
<i>M. indica</i> peels	positive	positive
<i>N. lappaceum</i> peels	positive	positive
<i>S. cumini</i> leaves	positive	positive
<i>S. melongena</i> fruit	positive	positive

The plant extracts were then tested for % total phenolic content using Folin-Ciocalteu method. *N. lappaceum* peels exhibited the highest % total phenolics with 40.70 % followed by *G. mangostana* pericarp with 29.00% and *S. cumini* peels with 14.30%.

Preliminary formulation studies were conducted on the six (6) plants that showed significant antioxidant activity namely *G. mangostana*, *C. longa*, *A. muricata*, *N. lappaceum*, *A. bunius* and, *F. vesca*. Product analysis for their antioxidant activity was conducted and presented in Tables 3 and 4. Formulations 4 and 5 exhibited the highest % total phenolic content with 10.5% and 10.3%,

respectively. These two formulations were then subject to DPPH assay to determine its radical scavenging activity. The assay yielded promising results. Formulation 4 showed 88.3% radical scavenging activity at 223 mg/kg tablet concentration while Formulation 5 exhibited 89.8% radical scavenging activity at 232 mg/kg tablet concentration.

Table 3: Results of the total phenolic content of the plant extracts expressed as gallic acid.

Plant Extract	% Total Phenolics (as Gallic acid)
<i>A. muricata</i> leaves	10.40
<i>C. grandis</i> peels	5.01
<i>C. longa</i> rhizomes	11.00
<i>G. mangostana</i> pericarp	29.00
<i>F. vesca</i> fruit	1.63
<i>M. indica</i> peels	11.70
<i>N. lappaceum</i> peels	40.70
<i>S. cumini</i> leavess	14.30
<i>S. melongena</i> fruit	1.75

Table 4: Results of the total phenolic content of the natural antioxidant chewable tablets.

Natural Antioxidant Formulation	Total Phenolics as Gallic Acid, % w/w
Formulation 1 (<i>G. mangostana</i> : <i>C. longa</i>)	4.65
Formulation 2 (<i>A. muricata</i> : <i>G. mangostana</i>)	4.30
Formulation 3 (<i>C. longa</i> : <i>A. muricata</i>)	2.49
Formulation 4 (<i>G. mangostana</i> : <i>N. lappaceum</i> : <i>F. vesca</i>)	10.5
Formulation 5 (<i>N. lappaceum</i> : <i>A. bunius</i> : <i>G. mangostana</i>)	10.3

Table 5: Ingredients of chewable tablet Formulation 4.

Ingredients	Formula 4 (%w/w)
Starch (glidant/disintegrant)	44.7
Stearic acid (binder)	12.2
Sucrose (sweetener)	12.2
<i>G. mangostana</i> pericarp extract	9.8
<i>N. lappaceum</i> peel extract	14.7
<i>F. vesca</i> fruit extract	4.9
Bixin colorant	1.5

Table 6: Ingredients of chewable tablet Formulation 5.

Ingredients	Formula 5 (%w/w)
Starch (glidant/disintegrant)	44.7
Stearic acid (binder)	12.2
Sucrose (sweetener)	12.2
<i>G. mangostana</i> pericarp extract	9.8
<i>N. lappaceum</i> peel extract	14.7
<i>A. bunius</i> fruits and leaves extract	4.9
Bixin colorant	1.5

Table 7: Results of the radical scavenging activity of the two (2) formulations with the highest % total phenolic content.

Natural Antioxidant Chewable Tablet	DPPH Radical – Scavenging Activity, %
Formulation 4 (<i>G. mangostana</i> : <i>N. lappaceum</i> : <i>F. vesca</i>)	88.3 (at concentration of 223 mg/kg)
Formulation 5 (<i>N. lappaceum</i> : <i>A. bunius</i> : <i>G. mangostana</i>)	89.8 (at concentration of 232 mg/kg)

Discussions

All plant extracts that were tested indicated the presence of flavonoids. Flavonoids consist of a large group of polyphenolic compounds having a benzo- γ -pyrone structure and are ubiquitously present in plants. It possesses many biochemical properties, but the best described property of almost every group of flavonoids is their capacity to act as antioxidants [3]. Flavonoids protect lipids against oxidative damage and inhibits free radical generation [4-7]. The plant extracts were then tested for % total phenolic content to further evaluate their antioxidant activity. Phenolics hinder oxidative degradation of lipids and thereby enhance the excellence and nutritional value of food. Furthermore, phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability of modifying gene expression [8,9]. Six (6) plants were used for the formulation of natural antioxidant supplement. *N. lappaceum* peels exhibited the highest phenolic content. This finding can be supported by a study conducted to evaluate the antioxidant and antibacterial activity of methanolic, ether and aqueous extract of *N. lappaceum* peels and seeds. Results from the said study suggested that the methanolic fraction of the *N. lappaceum* peels exhibited the highest antioxidant activity [10]. As for the *G. mangostana* pericarp, it showed 29.00% total phenolic content. Several studies were conducted that confirms the presence of antioxidant compounds in *G. mangostana* pericarp [11-13]. *C. longa* also exhibited significant phenolic content which can be verified by the past studies conducted regarding its antioxidant effect [14-17]. Likewise, *A. muricata* leaves showed remarkable phenolic content. One study found out that *A. muricata* possesses potent *in vitro* antioxidant activity as compared to other *Annona* species, highly suggesting its role as an effective radical scavenger [18]. Other studies confirm *A. muricata*'s effectivity as a natural antioxidant

[19,20]. Meanwhile, *F. vesca* showed lower total phenolic content compared to other plant extracts. But when formulated in combination with *G. mangostana* and *N. lappaceum*, it yielded remarkable radical scavenging activity by DPPH assay. Other studies showed that *F. vesca* contains high total antioxidant capacity content [21]. Moreover, *A. bunius* was used in Formulation 5 to produce a chewable tablet antioxidant supplement which also exhibited high radical scavenging activity. A study was able to isolate and identify six (6) phenolic compounds from *A. bunius* that supports its antioxidant activity [22].

Conclusion

In the present study, different plant materials were processed and subjected to antioxidant studies for the preparation of natural antioxidant dietary supplements. Results suggests that the twelve (12) plants studied namely, *F. vesca*, *S. melongena*, *N. lappaceum*, *M. indica*, *A. bunius*, *B. rubra*, *G. mangostana*, *S. cumini*, *D. alata*, *C. grandis*, *A. muricata* and *C. longa* have antioxidant capacity indicated by the presence of flavonoids and leucoanthocyanins when subjected to qualitative phytochemical analysis and by their total phenolic contents expressed as gallic acid. The results also imply that the combination of these plants to formulate natural antioxidant health supplement in the form of capsule and chewable tablet yielded remarkable radical scavenging activity. Use of natural products has been long encouraged because it has no or minimal side effects, in addition, it is cheap and environment-friendly. Thus, this study holds significance in using medicinal plants as a source for alternative medicine to prevent severe diseases.

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