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Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants

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The crude methanol extracts of four Philippine medicinal plants namely *Brucea amarissima* (Lour.) Merr. Bark, *Intsia bijuga* (Coebr.) O. Kuntze, *Laportea meyeniana* Warb, and *Pipturus arborescens* (Link) C.B. Rob leaves were examined for their antioxidant (radical scavenging) activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, and cytotoxicity using brine shrimp lethality assay (BSLA). LC_{50} values for BSLA ranged from 37.7 to 89.5 µg/ml, with *B. amarissima* having the lowest value and therefore the most potent, and *L. meyeniana* having the highest value. EC_{50} values for DPPH radical scavenging activity ranged from 343 to 1846 µg/ml, with *B. amarissima* and *I. bijuga* having the lowest and highest values, respectively. Phytochemical screening revealed the presence of flavonoids, tannins, triterpenes, steroids, anthraquinones, anthrones, flavonoid glycosides, and coumarins, which could be responsible for the bioactivities shown by these plants.

Key words: Philippine medicinal plants, DPPH radical scavenging activity, brine shrimp lethality assay, phytochemical screening.

INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Nearly 50% of drugs used in medicine are of plant origin, and only a small fraction of plants with medicinal activity has been assayed. There is therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them. The phytochemicals isolated are then screened for different types of biological activity (Harborne, 1998). A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). This method provides a front-line screen that can be backed up by more specific and expensive bioassays once the active compounds have been isolated. It appears that BSLT is predictive of cytotoxicity and pesticidal activity (Ghisalberti, 1993). Cytotoxicity via the brine shrimp lethality test is studied in order to reveal new anticancer compounds. Taxol ™, a new antitumor drug approved by FDA for treatment of ovarian, breast and non-small-cell lung carcinomas and originally isolated from the bark of *Taxus brevifolia* (He et al., 2001), was discovered in this way. Alternatively, crude plant extracts can be first assayed for particular activities and the active fractions then analyzed phytochemically (Harborne, 1998). A variety of bioassays are now available for the phytochemist to use in such work (Hostettmann, 1991). Recently, the DPPH radical scavenging assay has become popular in natural antioxidant studies because of its simplicity and high sensitivity.

This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH· (a stable and commercially available organic nitrogen radical) in test samples. Numerous studies on antioxidants present in plants have been conducted using the DPPH assay,

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including fruits and vegetables, medicinal plants, cereals and beans, spices and herbs, and teas and leaves. Studies on the antioxidant activities of alga and mushroom were also performed using this method (Moon and Shibamoto, 2009; Zahra et al., 2007). In the Philippines, medicinal plants are considered to be one of its natural living treasures. There are around 1,500 medicinal plants from the Philippines' 13,500 plants species of which more than 3,500 are considered indigenous. Only 120 medicinal plants have been scientifically validated for safety and efficacy (Galvez, 2003). The use of medicinal plants for primary health care is a substantial help to developing countries like the Philippines in meeting their drug requirements. Medicinal plant use however, has been based mostly on empirical grounds. There is a need for scientific validation of such empirical knowledge (Philippine Council for Health Research and Development, 1991). Four Philippine medicinal plants of interest to the authors include *Brucea amarissima* bark, and leaves of *Laportea meyeniana*, *Intsia bijuga,* and *Pipturus arborescens*.

B. amarissima (Lour.) Merr. (Simaroubaceae), a synonym of *Brucea javanica* (L.) Merr. is a plant indigenous to China, India, Indonesia, and Vietnam. The fruits of this plant have been used in traditional medicine of Indonesia and China. In Indonesia, the fruit is known as "Buah Makassar" and has demonstrated antimalaria, antipyretic and homeostatic effects. In China, it is known as "Yah-Tan-Tze" and is used to treat malaria and amoebic dysentery, and as an insecticide and anti-cancer (Wagih et al., 2008). In the Philippines, it is known as "Bogo-bogo" (Visayan dialect). The fruit, which contains glycosides called quassinoids, is most effective as an amoebicide. It also has an antimalarial property and is used externally to treat vaginal trichomoniasis and various fungal infections (Tan, 1980). It is also used as poultice on boils, and to treat ringworm, whipworm, roundworm and tapeworm, scurf, centipede bites, hemorrhoids, and enlarged spleen. The seed and seed oil of "Bogo-bogo" have been used in the treatment of warts and corns. It is marketed as Fructus Bruceae, which consists of dried ripe fruits of *B. javanica* (L.), and contains bruceosides and related quassinoids (WHO, 1999). In other countries, the bark or root bark of *B. amarissima* is a folk remedy for dysentery and verrucous tumor or cancer (Chang and But, 1987). To the best of our knowledge there has been no reported study on the bark of *B. amarissima*.

I. bijuga (Coebr). O. Kuntze (Leguminosae), locally known as "Ipil" in the Philippines, is a tree that grows in mangroves and coastal areas to about 50 m tall. Its timber, called "Merbau", is highly valued because it is stronger than teak and resistant to termites. In the Philippines, "Merbau" is used as a standard against which the durability of other timbers is assessed. It is also used for furniture making and carving woodcraft. The tree was extensively exploited in Southeast Asia for its timber that few natural strands remain. It is not abundant in Peninsular Malaysia, is considered rare and endangered in Singapore, listed as threatened in Indonesia, vulnerable in the Philippines, and almost extinct in Sabah. In the Philippines, the bark and leaves are used in traditional medicine for treating rheumatism, dysentery and urinary tract infection (Esperanza and Kitche, 2005) while the fruit is considered laxative (Quisumbing, 1978). In Madagascar, a decoction of the bark is used to treat diarrhea (Norsci*a* and Borgognini-Tarli, 2006). In Vanuatu, the inner bark is squeezed in coconut water and is taken as remedy for asthma, while the leaves or inner bark are squeezed with salt water and the solution is ingested for diabetes (Bradacs, 2008). The bark contains tannins, water soluble polymers, leucocyanidins, polyphenols, stilbenes, and polysaccharides while the heartwood contains mainly robinetin and small amounts of 3,5,4'-trihydroxystilbene (resveratrol), dihydromyricetin, myricetin and naringenin (Bandarayanake, 2002; Hills and Yazaki, 1973; Rollet, 1981). There were no previous reports on phytochemical screening and evaluation of antioxidant and cytotoxicity activities using DPPH and brine shrimp lethality assays, respectively, or isolation of bioactive compounds from *I. bijuga* leaves, hence the interest of the authors.

L. meyeniana Warb., locally known as "Alingatong" in the Visayan dialect in the Philippines, is a shrub or a small tree that grows to a height of 3 - 5 m. It belongs to the Urticaceae (Nettle) family which is characterized by the presence of stinging hairs known for causing contact dermatitis. Plants in this family have numerous uses. The stem fiber of some genera and species is of high quality and is used to make cloth, fishing nets and ropes and for some industrial materials. Members of the genus Urtica are known for the stinging sensation caused by the injection of histamine, acetylcholine, and 5-hydroxytryptamine into the skin by fine, needle-like projections found on the leaves and stems of the plants. Despite this deterrent, many of these nettle species have been used for generations in the preparation of herbal medications. In Butuan City, Philippines, the extract of the root of *L. meyeniana* is used as a cure for ringworm (Esperanza and Kitche, 2005). Dried or powdered leaves are used to stop bleeding while a decoction of the leaves is used for nosebleeds. To the best of our knowledge, there has been no reported study on the leaves of *L. meyeniana.*

P. arborescens (Link) C.B. Rob (Urticaceae), locally known as "Handalamay", is a small tree which is common and widely distributed in the Philippines. It also occurs in Borneo, and Botel Tobago. The scrapings of the bark are used externally as a cataplasm for boils, while the leaves are used to cure herpes (Esperanza and Kitche, 2005). *P. arborescens* is also used as remedy for boils and skin diseases (Quisumbing, 1978). From the hexane extract of

the leaves, triterpenes such as glutinone, friedelin, and glutinol, and a mixture of common sterols such as campesterol, stigmasterol and sitosterol were isolated (Gabona, 2000). A pure isolate active against *Bacillus subtilis* was obtained from the crude ethyl acetate extract of the leaves after rechromatography (Rosal, 1995). There has been no reported study on the phytochemical screening and/or antioxidant activity on the leaves of *P. arborescens.* In our continuing search for bioactive compounds from local medicinal plants, the methanol extracts of the above-mentioned four Philippine medicinal plants were evaluated for their antioxidant (radical scavenging) and cytotoxic activities using DPPH and brine shrimp lethality assays, respectively. Furthermore, phytochemical screening of the methanol extracts using test tube and thin - layer chromatography (TLC) methods were performed.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used (various brands) were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical was purchased from Wako Chemical Co., Tokyo, Japan.

Plant materials

The plants were collected from different localities of Mindanao, Philippines in 2007 and 2008 and identified by Prof. Josefa D. Villanueva of the Department of the Biological Sciences, Mindanao State University - Iligan Institute of Technology, Iligan City, Philippines. Fresh plant materials were washed under running tap water, air-dried for three to four weeks, homogenized to fine powder, and stored in airtight bottles.

Plant extraction

Powdered air - dried plant samples were soaked in methanol for 48 h, filtered, and the solvent removed *in vacuo* using a rotary evaporator at a temperature below 40°C. Extracts were stored in sample bottles in the refrigerator prior to use.

Phytochemical screening

Phytochemical screening of the crude methanol extract of the four plants was carried out using standard phytochemical methods described by Harborne (1998) and Aguinaldo et al., (2005).

Brine shrimp lethality assay

The assay was carried out according to the principle and protocol previously described by Meyer et al. (1982), McLaughlin et al. (1991), and Krishnaraju et al. (2005), with slight modifications. Brine shrimp eggs (*Artemia salina*) were placed on one side of a small tank which was filled with boiled, filtered sea water, covered with aluminum foil, and fully aerated. After 48 h incubation at room temperature and under illumination, the resulting nauplii (larvae) were attracted to the other side of the tank with a light source and collected with a Pasteur pipette. Samples for testing were prepared by initially dissolving 50 mg of crude extract in 5 mL of dimethyl sulfoxide (DMSO) and further diluted with sea water to produce the required concentrations. Appropriate amounts (500 -, 50 -, or 5 - µL for 1000, 100, and 10 µg/mL, respectively) were transferred to vials containing small filter paper discs, air-dried overnight to evaporate the solvent, and further dried under nitrogen gas. Ten brine shrimps were transferred to each sample vial, and boiled, filtered sea water was added to make 5 mL. Tests for each concentration were done in triplicate. A control experiment containing 500 -, 50 - , or 5- µL DMSO in 5 mL of boiled, filtered sea water and ten brine shrimps was also performed in triplicate for each concentration. The vials were maintained under illumination. Survivors were counted after 24 h and the percentage mortality at each vial and control was determined using the equation:

$$\% \text{ mortality} = (\text{no. of dead nauplii/ initial no. of live nauplli}) \times 100$$

Probit analysis by Finney (1971) was used to determine the concentration at which lethality to brine shrimp represents 50% (LC_{50}). LC_{50} values less than 100 ppm (or 100 µg/mL) were considered significant (Gupta, 1996).

DPPH radical scavenging activity

Free radical scavenging capacity was evaluated on the basis of the scavenging activity of DPPH by measuring the reduction of absorbance at 517 nm. The method was carried out as described by Brand-Williams et al. (1995), Mothana et al. (2008), and Spanou et al. (2008), with slight modifications. Crude methanol extract of four Philippine medicinal plants were redissolved in methanol and various concentrations (10, 50, 100, 500 and 1000 µg/mL) of each extract were prepared. The assay mixture contained in a total volume of 1 mL consists of 500 µL of the extract, 125 µL of freshly prepared DPPH solution (1mM in methanol) and 375 µL of solvent (methanol). The contents were mixed vigorously in a vortex mixer for 10 s and incubated at room temperature in the dark (wrapped with Aluminum foil) for 30 min. The absorbance was read at 517 nm using a spectrophotometer (Fischer-Scientific, Spectro Master Model 415). In each experiment, the tested sample alone in methanol was used as blank while the DPPH solution alone in methanol was used as control. All experiments were carried out in triplicate. L-ascorbic acid was used as a standard. The radical scavenging activity of samples corresponded to the intensity of quenching DPPH. The results were expressed as percentage inhibition.

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}})/ A_{\text{control}}] \times 100$$

where A_{control} and A_{sample} are the absorbance values of the control and test sample, respectively. The effective concentration of sample required to scavenge DPPH radical by 50% (EC_{50}) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentration.

RESULTS

Phytochemical screening

Phytochemical screening on the crude methanol extracts of four Philippine medicinal plants namely *B. amarissima*, *I. bijuga*, *L. meyeniana* and *P. arborescens* were done

Table 1. Results of phytochemical screening on crude methanol extracts of four Philippine medicinal plants.

Phytochemicals	*B. amarissima* bark	*I. bijuga* leaves	*L. meyeniana* leaves	*P. arborescens* leaves
Anthrones	+	+	+	+
Flavonoids	+	+	+	+
Glycosidic flavonoids	+	+	+	+
Phenolic compounds	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Triterpenes	+	+	+	+
Alkaloids	-	-	-	-
Anthraquinones	-	+	+	+
Coumarins	-	+	-	+

+ = Present; - = Absent.

Table 2. Results of Brine Shrimp Lethality Assay on crude methanol extracts from four Philippine medicinal plants.

Plant	% Mortality at Different Concentrations *			LC_{50} , 24h h
	10 µg/ml	100 µg/ml	1000 µg/ml	µg/ml
B. amarissima	20.0	60.0	100.0	37.7
I. bijuga	3.3	23.3	100.0	86.5
L. meyeniana	6.7	10.0	100.0	89.5
P. arborescens	13.3	33.3	100.0	57.5

* mean of 3 determinations.

using test tube and TLC methods. Results (Table 1) revealed the presence of secondary metabolites such as anthrones, flavonoids, glycosidic flavonoids, phenolic compounds, steroids, tannins, triterpenes, anthraquinones, and coumarins. No alkaloids were detected in all of the extracts. Phytochemicals such as anthrones, flavonoids, glycosidic flavonoids, phenolic compounds, steroids, tannins and triterpenes were present in all of the crude methanolic extracts. Anthraquinones were present in all extracts except *B. amarissima* bark, while coumarins were present in *I. bijuga* and *P. arborescens* leaves only.

Brine shrimp lethality assay

The results of BSLA on crude methanol extracts of the four Philippine medicinal plants (% mortality at different concentrations and LC_{50} values) are shown in Table 2. The percentage mortality increased with an increase in concentration. All of the extracts showed 100.0% mortality to brine shrimp at 1000 µg/mL. LC_{50} values ranged from 37.7 to 89.5 µg/mL, with *B. amarissima* bark *having* the lowest value (most potent); this was followed by *P. arborescens* leaves (57.5 µg/mL), then by *I. bijuga* leaves (86.5 µg/ml) and lastly *L. meyeniana* leaves (89.5 µg/ml).

DPPH radical scavenging activity

The results of DPPH free radical scavenging activity on the four crude methanol extracts and of L-ascorbic acid (standard) are shown in Table 3. The highest radical scavenging activity (EC_{50} value, 343 µg/ml) was shown by *B. amarissima*; this was followed by *P. arborescens*, and *L. meyeniana*, with EC_{50} values of 838 and 920 µg/ml, respectively. The lowest radical scavenging activity was shown by *I. bijuga* (EC_{50} value of 1846 µg/ml). These results are not comparable to L-ascorbic acid (EC_{50} value of 2 µg/ml).

DISCUSSION

Phytochemical screening

Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods available which

Table 3. DPPH free radical scavenging activity of crude methanol extracts of four Philippine medicinal plants.

Plant / Standard	% Inhibition at different concentration*					EC_{50}
	10 µg/mL	50 µg/mL	100 µg/mL	500 µg/mL	1000 µg/mL	µg/mL
B. amarissima	18.7 ± 2.9	22.8 ± 7.2	29.9 ± 4.3	78.9 ± 3.1	95.8 ± 0.4	343
I. bijuga	5.7 ± 5.3	9.3 ± 3.8	13.6 ± 0.8	23.2 ± 1.0	29.4 ± 3.2	1846
L. meyeniana	3.2 ± 0.9	8.2 ± 0.9	10.8 ± 2.0	25.0 ± 1.8	55.7 ± 0.7	920
P. arborescens	8.9 ± 1.2	9.5 ± 1.0	13.7 ± 3.2	31.7 ± 1.0	58.8 ± 8.8	838
*L. ascorbic acid***	77.4 ± 3.1	98.1 ± 0.2	98.3 ± 0.2	97.2 ± 0.2	98.0 ± 0.1	2

*Data are expressed as the mean of triplicate ± SD.
**Standard; Values for % inhibition at 2.5, 5.0 and 25 µg/mL are 48.3 ± 3.4, 54.6 ± 5.4, and 97.6 ± 0.1, respectively.

eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important. Thin-layer chromatography (TLC) is the simplest and cheapest method of detecting plant constituents because the method is easy to run, reproducible and requires little equipment (Marston et al., 1997).

Anthrones, which were present in all of the crude plant extracts examined, may act as topical agents in the treatment of psoriasis (Wiegrebe and Muller, 1995) and as naturally occurring competitive inhibitors of adenosine-triphosphate-citrate lyase (ACL), a liver enzyme which catalyzes the formation of acetyl-COA for fatty acid and cholesterol biosynthesis. Inhibition of ACL offers a potentially unique way to control plasma cholesterol and triglyceride levels (Oleynek et al., 1995). Together with anthraquinones, anthrones exhibited antioxidant and radical scavenging effects (Malterud et al., 1993) and antiviral activity against human cytomegalovirus (Barnard et al., 1992).

Anthraquinones also possess antiparasitic (Pieters and Vlietinck, 2005), bacteriostatic, antidepressant, and antimicrobial properties (Cowan, 1999). The folkloric use of *P. arborescens* leaves for treating herpes simplex and skin diseases may be confirmed by the presence of anthrones and anthraquinones. Tannins are polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency (Cowan, 1999). They are used in pharmaceutical preparations because of their astringent action. Tannins are known to possess general antimicrobial and antioxidant activities (Riviere et al., 2009). At low concentration tannins can inhibit the growth of microorganisms, and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism (Adekunle and Ikumapayi, 2006). Recent reports show that tannins may have potential value as cytotoxic and/or antineoplastic agents (Aguinaldo et al., 2005). Aside from the use of tannins as antimicrobial agents or prevention of dental caries, they are now being used in the manufacture of plastics, paints, ceramics and water softening agents (Bandarayanake, 2002). The presence of tannins in all of the crude extracts examined may justify their therapeutic use as astringent (*B. amarissima* bark), to stop bleeding (*L. meyeniana* leaves), and to cure dysentery (*I. bijuga* leaves) and boils (P*. arborescens* leaves).

Flavonoids, (a large group of naturally occurring plant phenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones), also known as nature's tender drugs, possess numerous biological/ pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcenogenic, hepatoprotective, and cytotoxic activities of flavonoids have generated interest in studies of flavonoid-containing plants. Of these biological activities, the anti-inflammatory capacity of flavonoids has long been utilized in Chinese medicine and the cosmetic industry as a form of crude plant extracts (Aguinaldo, et al., 2005; Moon YJ et al, 2006; Veitch, 2007; Jiang H et al., 2008; Kim HP et al, 2004; Wu J-H et al., 2008). The presence of flavonoids in all crude plant extracts may confirm their folkloric use in treating rheumatism (*B. amarissima* bark) and in curing herpes simplex (*P. arsborescens* leaves). All of the crude plant extracts examined may be potential source of triterpenes.

Triterpenoids have a range of unique and potentially usable biological effects and reference to the use of plants with high saponin / triterpenoid content can be found in the first written herbarium. Here alluded to are plants (*Panax ginseng*, *Ganoderma lucidum*, *Platycodon grandiflorum*, Indian frankincense-resin from the tree *Boswellia serrata*) that were highly prized as panaceas *par excellence* due to their wide-ranging effects. Triterpenoids are studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. They are used in the prevention and treatment of hepatitis,

parasitic and protozoal infections and for their cytostatic effects. The disadvantage of using triterpenoids is the toxicity associated with their hemolytic and cytostatic properties. Hand in hand with ongoing extraction and isolation of natural products therefore, is the development of synthetic derivatives with lower toxic and higher therapeutic potential (Dzubak et al., 2006).

Coumarins, phenolic substances made of fused benzene and α-pyrone rings, have long been recognized to possess anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. The hydroxycoumarins are typical phenolic compounds and, therefore act as potent metal chelators and free radical scavengers (Kostova, 2005). Only two plant extracts tested positive for the presence of coumarins: *I. bijuga* and *P. arborescens* leaves. The above-mentioned results show that the four Philippine medicinal plants examined may be rich sources of phytochemicals particularly flavonoids, triterpenes, steroids, tannins, anthraquinones, anthrones and coumarins, which can be isolated and further screened for different kinds of biological activities, depending on their reported ethnobotanical and/or therapeutic uses. Quantitative analyses of these phyto-chemicals may also be done to guide the researchers on which particular bioactive class of compounds may be subjected to subsequent target isolation.

Brine shrimp lethality assay

The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials. Brine shrimp nauplii have been previously utilized in various bioassay systems. Among these applications have been the analyses of pesticidal residues, mycotoxins, stream pollutants, anesthetics, dinoflagelate toxins, morphine-like compounds, carcinogenicity of phorbol esters and toxicants in marine environment. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay (McLaughlin et al., 1991; Meyer et al., 1982; Sam TW, 1993).

The variation in BSLA results (Table 2) may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts. Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or µg/mL) to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. BSLA results may be used to guide the researchers on which crude plant extracts/fractions to prioritize for further fractionation and isolation of these bioactive compounds. Other cytotoxicity tests and specific bioassays may be done on the isolated bioactive compounds later.

DPPH radical scavenging activity

Antioxidants have been widely used in the food industry to prolong shelf life. However, there is a widespread agreement that some synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene (BHA and BHT, respectively) need to be replaced with natural antioxidants because of their potential health risks and toxicity. Thus, the search for antioxidants from natural resources has received much attention, and efforts have been made to identify new natural resources for active antioxidant compounds (Dudonne et al., 2009). Phenolic natural products such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals and inhibiting peroxidation. Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer, and inflammatory disorders (Cioffi et al., 2002). In addition, these naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage from occurring in the body. One way of estimating antioxidant activity is by the use of the stable free radical DPPH (Molyneux, 2004; Brand-Williams et al., 1995; Dudonne et al., 2009; Moon and Shibamoto, 2009). Results (Table 3) show that the activities of the crude methanol extract of the four Philippine medicinal plants are not comparable to L-ascorbic acid. This is understandable, since L-ascorbic acid is already in a pure form, while the crude plant extracts still need to be processed in order to isolate the compounds responsible for their antioxidant activity. However, this assay may be used to guide the fractionation and isolation of potential antioxidant compounds from these plant extracts.

Of the four Philippine medicinal plants examined, *B. amarissima* bark appears to be the most promising source of cytotoxic and antioxidant compounds. As shown in Tables 2 and 3, *B. amarissima* gave the lowest LC_{50} and EC_{50} values for BSLA and DPPH radical scavenging activity, respectively. This is in agreement with the initial findings of a study done in Baylor College of Medicine, Texas, USA (A. Daquinag, personal communication) where *B. amarissima* (crude ethanol -, methanol - and chloroform - soluble) extracts were shown to be active against HeLa cells. Another promising source of useful bioactive compounds is the *P. arborescens* leaves, which gave the second to the lowest LC_{50} and EC_{50} values for BSLA and DPPH radical scavenging activity, respectively (Tables 2 and 3). Similarly, the chloroform -, methanol -, and ethanol -soluble extracts of this plant were also found to be active against HeLa cells

(A. Daquinag, personal communication). In the same study of A. Daquinag, the ethanol -, chloroform -, and hexane -soluble extracts of *I. bijuga* leaves also gave positive results for activity against HeLa cells. No study was done on *L. Meyeniana* leaves regarding activity against Hela cells but results from Tables 1, 2 and 3 show that it can also be a potential source of cytotoxic and antioxidant compounds. Studies on the isolation, structure elucidation and biological activities of the components of these four Philippine medicinal plants are now on-going at the Chemistry Department, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines.

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REFERENCES

Adekunle AA, Ikumapayi AM (2006). Antifungal Property and Phytochemical Screening of the Crude Extracts of *Funtumia elastica* and *Mallotus oppositifolius*. West Indian Med. J. 55(6): 219-223.

Aguinaldo AM, Espeso EI, Guevara BQ, Nonato MG (2005). Phytochemistry. In: Guevara BQ (ed.) A Guidebook to Plant Screening: Phytochemical and Biological. University of Santo Tomas, Manila, Philippines.

Bandarayanake WM (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl. Ecol. Manage. 10(6): 421-452.

Barnard DL, Huffman JH, Morris JL, Wood SG, Hughes BG, Sidwell RW (1992). Evaluation of the antiviral activity of anthraxquinones, anthrones and anthraquinone derivatives against human cytomegalovirus. Antiviral – Res. 17(1): 63-77.

Bradacs G (2008). Ethnobotanical Survey and Biological Screening of Medicinal Plants from Vanuatu. Ph D dissertation, der Universitat Regensburg, Frankfurt, Germany.

Brand-Williams W, Cuevelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. u-Technol. 28: 25-30.

Chang HM, But PP (1987). "Yadanzi". Pharmacology and Applications of Chinese Materia Medica. Vol II. World Scientific Pub. Co., Singapore.

Cioffi G, D'Auria M, Braca A, Mendez J, Castillo A, Morelli I, De Simone F, De Tommasi N (2002). Antioxidant and Free Radical Scavenging Activity of Constituents of the Leaves of *Tachigalia paniculata*. J. Nat. Prod. 65: 1526-1529.

Cowan MM (1999). Plant Products as Antimicrobial Agents. Clin. Microbiol. Rev. 12(4): 564-582.

Dudonne S, Vitrac S, Coutiere P, Woillez M, Merillon JM (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD and ORAC assays. J. Agric. Food Chem. 57: 1768-1774.

Dzubak P, Hajduch M, Vydra D, Hustova A, Kvasnica M, Biedermann D, Markova L, Urba M, Sarek J (2006). Pharmacological activities of natural triterpenoids and their therapeutic implications. Nat. Prod. Rep. 23: 394-411.

Esperanza LM, Kitche GO (2005). Inventory of medicinal tree species in the secondary growth forest of Sitio Tagkiling, Anticala, Butuan City as utilized by the locals. 2005 NORMISIST Research and Development In-House Review.Northern Mindanao State of Science and Technology, Butuan City, Philippines.

Finney DJ (1971). Probit Analysis. 3rd ed. Cambridge University Press, Cambridge.

Gabona MG (2000). Triterpenoids and Other Metabolites from the Hexane Extract of *Pipturus arborescens*. MS Thesis, De La Salle University, Manila, Philippines.

Galvez JZ (2003). The need for national colloquium on medicinal plants research and business opportunities: Proceedings of the seminar on the State of the Art of Medicinal Plant Research and Business Opportunities. Manila, Philippines.

Ghisalberti EL (1993). Detection and Isolation of Bioactive Natural Products. In: Colgate SM and Molyneux RJ (eds.) Bioactive Natural Products: Detection, Isolation and Structural Determination. CRC Press, USA.

Gupta MP, Monge A, Karikas A, Lopez de Cerain A, Solis PN, de Leon E, Trujillo M, Suarez O, Wilson F, Montenegro G, Noriega Y, Santana AI, Correa M, Sanchez C (1996). Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA intercalation. Int. J. Pharmacol. 34(1): 19-27.

Harborne JB (1998). Phytochemical Methods. Chapman and Hall, London.

He L, Orr GA, Horwitz SB (2001). Novel molecules that interact with microtubules and have functional activity similar to Taxol ™ Drug Discovery Today 6(22): 1153-1164.

Hills WE, Yazaki Y (1973). Polyphenols of Intsia heartwoods. Phytochemistry 12: 2491-2495.

Hostettmann K (ed.) (1991). Methods in Biochemistry, Vol. 6, Assays for Bioactivity, Academic Press, London.

Jiang H, Zhan WQ, Liu X, Jiang SX (2008). Antioxidant activities of extracts and flavonoid compounds from *Oxytropis falcate* Bunge. Nat. Prod. Res. 22(18): 1650-1656.

Kim HP, Son KH, Chang HW, Kang SS (2004). Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. J. Phamacol. Sci. 96: 229-245.

Kostova I (2005). Synthetic and Natural Coumarins as Cytotoxic Agents. Curr. Med. Chem.- Anti-Cancer Agents 5(1): 29-46.

Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. Int. J. Appl. Sci. Eng. 3(2): 125-134.

Malterud KE, Farbrot TL, Huse AE, Sund RB (1993). Antioxidant and radical scavenging effects of anthraquinones and anthrones. Pharmacology 47(Suppl. 1): 77-85.

Marston A, Maillard M, Hostettmann K (1997). GIT Laboratory J. I: 36-39.

McLaughlin J, Chang C, Smith D (1991). Proceedings of the 18th National Seminar and Unesco Workshop on Natural Products. Institute of Advance Studies, University of Malaysia, Malaysia.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982). Brine Shrimp: A convenient general bioassay for active plant constituents. Planta Med. 45: 31-34.

Molyneux P (2003). The use of the stable free radical diphenylpicrylhydrazy (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 26(2): 211- 219.

Moon JK, Shibamoto T (2009). Antioxidant assays for plant and food components. J. Agric. Food Chem. 57(5): 1655-1666.

Moon YJ, Wang X, Morris ME (2006). Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. Toxicol. *In vitro* 20: 187-210.

Mothana RA, Abdo SAA, Hasson S, Althawab FMN, Alaghbari SAZ,

Lindequist U (2008). Antimicrobial, antioxi-dant and cytotoxic activities and phytochemical screening of some Yemeni medicinal plants. eCAM: 1-8

Norscia I, Borgognini-Tarli SM (2006). Ethnobotanical reputation of plant species from two forests of Madagascar: A preliminary investigation. S. Afr. J. Bot. 72: 656-660.

Oleynek JJ, Barrow CJ, Burns MP, Sedlock DM, Murphy DJ, Kaplita PV, Sun HH, Copper R, Gilluma AM, Chadwick CC (1995). Anthrones, naturally occurring competitive inhibitors of adenosine-triphosphate-citrate lyase. Drug Dev. Res. 36(1): 34-42.

Pieters L, Vlietinck AJ (2005). Bioguided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds? J. Ethnopharmacol. 100: 57-60.

Philippine Council for Health Research and Development (1991). Selection and Scientific Validation of Medicinal Plants for Primary Health Care. Manila, Philippines.

Quisumbing E (1978). Medicinal Plants of the Philippines. Katha Publishing Co., Philippines.

Rievere C, Van Nguyen TH, Pieters L, Dejaegher B, Heyden YV, Minh CV, Quetin-Leclercq J (2009). Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. Phytochemistry 70: 86-94.

Rollet B (1981). Bibliography on mangrove research 1600-1975. UNESCO Paris. Pub. Information Retrieval Ltd., London p. 479.

Rosal RR (1995). Bioassay-guided isolation and structure elucidation of some metabolites from *Pipturus arborescens*. MS Thesis, De La Salle University, Manila, Philippines.

Sam TW (1993). Toxicity testing using the brine shrimp: *Artemia salina*. In: Colgate SM, Molyneux RJ (eds.). Bioactive Natural Products: Detection, Isolation and Structural Determination. CRC Press, USA.

Spanou C, Bourou G, Dervishi A, Aliguiannis N, Angelis A, Komiotis D, Skaltsounis AL, Kouretas D (2008). Antioxidant and chemopreventive properties of polyphenolic compounds derived from Greek legume plant extracts. J. Agric. Food Chem. 56(16): 6967-6976.

Tan, ML (1980). Philippine Medicinal Plants in Common Use: Their Phytochemistry and Pharmacology. AKAP, Quezon City, Philippines.

Veitch NC (2007). Isoflavonoids of the Leguminosae. Nat. Prod. Rep. 24: 417-464.

Wagih ME, Atam G, Wiryowidagdo S, Attia K (2008). Improved production of indole alkaloid cathin-6-one. Indian J. Sci.Technol. 1(7): 1-6.

Wiegrebe W, Muller K (1995). Treatment of psoriasis with anthrones – chemical principles, biochemical aspects and approaches to the design of novel derivatives. Skin Pharmacol. 8: 1-24.

World Health Organization (1999). WHO Monographs on selected medicinal plants. WHO, Geneva.

Wu JH, Tung YT, Chien SC , Wang SY, Kuo YH, Shyur LF, Chang ST (2008). Effect of Phytocompounds from the Heart-wood of *Acacia confusa* on Inflammatory Mediator Production. J. Agric. Food Chem. 56: 1567-1573.

Zahra R, Mehnarz M, Farzaneh V, Kohzad S (2007). Antioxidant activity of an extract from a brown alga, *Sargassum boveanum*. Afr. J. Biotechnol. 6(24): 2740-2745.