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The antioxidant properties of the Philippine medicinal plants *Cassia sophera* Linn., *Derris elliptica* Benth, *Ficus minahassea* Tesym. and De Vr., *Leea aculeata* Blume and *Leucosyke capitellata* Wedd

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Abstract. The search for natural antioxidants has received much attention and efforts have been made to identify and validate plant sources of these natural products. In this study, the antioxidant activities of the decoction, crude ethanol and 50:50 ethanol-water extracts from the leaves of *Cassia sophera* Linn., *Derris elliptica* Benth, *Ficus minahassea* Tesym. & De Vr., *Leea aculeata* Blume and *Leucosyke capitellata* Wedd were determined. The results obtained from the *in vitro* antioxidant screening of the plant leaf extracts using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, total phenolic contents by Folin-Ciocalteu method and phosphomolybdenum method provide good correlation coefficient (r = 0.83288). The highly ranked plant species in all methods used are from the decoction, crude ethanol and 50:50 ethanol-water extracts of the plant *L. capitellata* and *L. aculeate* with interestingly comparable antioxidant profiles with those of the reference standards ascorbic acid and butylated hydroxytoluene (BHT). Results indicate that natural antioxidants and free radical scavengers can be obtained from these species.

Key Words: Natural products, free radical scavengers, total phenolic content, phosphomolybdenum method, folkloric remedies.

Introduction. Plants are known to be rich in biologically active substances, many of which exhibit outstanding antioxidant activity which consequently may become a potential source of herbal drugs and supplements (Sati et al 2010; Brewer 2011; Kasote et al 2015). In the Philippines, plants with medicinal values are gaining popularity and getting recognition. To advance knowledge on specific plant chemical constituents, assays such as antioxidant screenings are being conducted to determine the actual value of folkloric remedies.

Cassia sophera Linn. is a plant belonging to the family Caesalpiniaceae. The juice of the plant's leaves are used for ringworm, externally used for washing syphilitic sores and dropped into ears invaded by insects (Aminabee & Lakshmana Rao 2012). Internally, it is used as expectorant for coughs while the decoction of the whole plant is used as expectorant in acute bronchitis (Hudaib et al 2008). The roots are used for snake bites (Hudaib et al 2008). *Derris elliptica* Benth is a member of a Fabaceae (pea) family. It has been used as a fish poison and as an effective pesticide (Starr et al 1999). Scrap of the root with a little opium is an abortifacient while infusion or decoction of roots with coconut oil may be applied to itchy lesions (Khan et al 2006). A plaster of the root is used for abscesses and leprosy (Wiwattanapatapee et al 2009). *Ficus minahassea* Tesym. & De Vr. belongs to the Moraceae/Ficeae family. The bark decoction has astringent properties (Koh et al 2009). The decoction of boiled roots in water is administered three times daily to enhance milk production in lactating mother; also for relief muscle pains or for fatigue in women (Olowa et al 2012). *Leea aculeata* Blume is a member of Vitaceae family, a species in the genus Leea. It is used in aid of fever, after childbirth, headache and used for poulticing (http://www.asianplant.net/Vitaceae/Leea_indica.htm). *Leucosyke capitellata*

(Prior) Wedd belongs to the Urticaceae family. Its root extract is a cure for phthisis, cough, headache and gastralgia (http://www.stuartxchange.com/Alagasi.html). This plant is believed to have potential to treat diabetes (Ahmad & Ismail 2003) with its leaf extracts which possess antihyperglycaemic effect evidenced in diabetic rats (Ling 2008).

Antioxidants have been shown to inhibit the propagation of free radical reactions, protect the human body from diseases like heart disease, stroke, arteriosclerosis, diabetes, and cancer (Tanizawa et al 1992; Hertog et al 1993; Duh 1998). Some studies showed that people with low intakes of antioxidant-rich fruits and vegetables were at greater risk for developing these chronic conditions (Pyrzynska & Pekal 2013). Plants are known to be rich in biologically active substances, many of which exhibit antioxidant activity (Krishnaiah et al 2011). Antioxidant activity screening provides information on potential plant material having specific and effective antioxidant properties. These plants can then be used to maintain cellular health (Gulcin 2012).

This work evaluated the antioxidant activities of the ethanol, 50:50 ethanol:water and water extracts of the leaves of the five plants of interest with the aim of bringing forward the continuing search for naturally-occurring antioxidants in plants.

Material and Method

Plant materials. Plant leaf samples were collected from as follows: *C. sophera* (Binuni, Demologan Bacolod Lanao del Norte, Philippines), *D. elliptica* (Pangangan, Bacolod, Lanao del Norte, Philippines), *F. minahassea* (Merila, Barangay Ubaldo Laya, Iligan City, Philippines), *L. aculeata* (Kibanggay Mount Kitanglad, Bukidnon, Philippines) and *L. capitellata* (Pandanan, Sultan Naga Dimaporo, Lanao del Norte, Philippines). The collected plant samples were authenticated and identified by Tabaranza A. E., a taxonomist from the Department of Biology, MSU-IIT, Iligan City, Philippines. These were thoroughly cleaned, air-dried for two or more weeks, and then homogenized into fine powder.

Plant extraction. The ground leaves (1 kg) of each plant sample were soaked separately with absolute ethanol (about 2.5 L) and 50:50 (1L:1L) ethanol-water for three days. The solution was filtered, concentrated under a rotary evaporator at ambient temperature and freeze-dried to yield the crude ethanol and aqueous extracts. The decoctions were prepared by boiling small pieces of fresh leaves (1 kg) of the plant samples for 15 minutes with sufficient amount of distilled water (1 kg : 2 L), cooled, filtered and freeze-dried.

DPPH radical scavenging assay. The DPPH radical scavenging activity of each of the test plant extracts was examined by comparison with that of known antioxidant butylated hydroxytoluene (BHT) as described by Lee & Shibamoto (2001). Four concentrations (25, 50, 100, and 500 ppm) were added with 3 mL of methanolic solution of DPPH (0.1 mM). The solution was shaken vigorously and then allowed to stand at room temperature for one hour. Three trials were done for each plant extract. Absorbance was measured at 517 nm against methanol as a blank in the spectrophotometer. 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 percent inhibition and concentration. The percent of DPPH discoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = \frac{A_{control} - A_{sample}}{A_{control}} x100$$

Total antioxidant activity assay. The total antioxidant activity of the extracts was evaluated by the phosphomolybdenum method as described by Prieto et al (1999). Briefly, 0.3 mL extract solution at 200 µg/mL was added with 3.0 mL reagent solution (6M H_2SO_4, 28 mM sodium phosphate, 4 mM ammonium molybdate), incubated at 95^{o}C

for 90 minutes and cooled to room temperature. Its absorbance was read at 695 nm using a spectrophotometer. Three trials were done for each test extract. The antioxidant activity of the test plant extracts were expressed as ascorbic acid equivalents (AAE) and butylated hydroxytoluene equivalents (BHTE) using their corresponding calibration curves.

Total phenolics content assay. The total phenolic content of each sample was determined by combining 0.1 mL (0.5 µg/mL) of the sample with 2.8 mL of 10% Na_2CO_3 and 0.1 mL of 2N Folin-Ciocalteu reagent as described in Makkar et al (1993). After 40 minutes, absorbance at 725 nm was measured. Total phenolics was determined as gallic acid equivalence (GAE) in milligrams per gram of sample by computing from a standard calibration curve constructed for different concentrations of gallic acid (25-200 mg g^{-1}).

Results and Discussion

DPPH radical scavenging assay. The DPPH radical scavenging activities of the plant extracts were examined by comparison with that of a known antioxidant BHT. The radical scavenging capacity resulted in a color change from purple to yellow, which was measured spectrophotometrically. The disappearance of the purple color was monitored after 1 h at 517 nm. The plant extracts' scavenging activities against the DPPH radical are shown in Table 1.

Table 1

DPPH radical-scavenging activities of the plant leaf extracts at various concentrations

Plant/ Standard	*Extracts*	*Antiradical activity, %**					*EC_{50}, ppm*
		Code	*25 ppm*	*50 ppm*	*100 ppm*	*500 ppm*	
C. sophera	Decoction	CsD	7.27	7.70	9.53	22.22	>500.00
	Ethanol	CsE	0.00	0.00	0.49	11.11	>500.00
	EtOH:H_2O	CsA	0.00	0.00	2.00	19.61	> 500.00
D. elliptica	Decoction	DeD	12.18	19.71	37.54	91.72	229.85
	Ethanol	DeE	0.00	1.54	5.51	30.79	>500.00
	EtOH:H_2O	DeA	2.11	6.66	14.55	71.60	350.05
F. minahassea	Decoction	FmD	12.33	12.54	17.63	39.47	>500.00
	Ethanol	FmE	1.61	3.72	5.51	30.79	>500.00
	EtOH:H_2O	FmA	2.11	6.66	14.55	71.60	426.71
L. aculeata	Decoction	LaD	22.22	34.92	59.77	93.40	149.02
	Ethanol	LaE	17.53	27.33	53.92	93.80	181.61
	EtOH:H_2O	LaA	29.86	51.95	83.15	92.40	40.45
L. capitellata	Decoction	LcD	6.88	8.81	17.31	64.18	445.84
	Ethanol	LcE	0.00	0.00	0.14	14.44	>500.00
	EtOH:H_2O	LcA	45.93	47.29	51.95	90.61	74.95
BHT	-	-	18.78	39.29	68.41	93.80	128.97

* - mean of triplicate analysis.

The results show that the radical-scavenging activities of all the extracts as well as those of the positive control were concentration-dependent. At the lowest concentration tested, the EtOH:H_2O extract of *L. capitellata* exhibited a higher activity than that of BHT. Furthermore, four plant extracts showed similar radical-scavenging potency as the control at 500-ppm concentration. These are the decoction of *D. elliptica*, the EtOH:H_2O extract of *L. capitellata* and all the extracts of *L. aculeata*. Accordingly, the effective median concentration (EC_{50}) values of two of these extracts are even lower than that of BHT. In fact, the results indicate that the EtOH:H_2O extract of *L. capitellata* has a

radical-scavenging activity that is three times more potent than that of the known antioxidant BHT.

Total antioxidant activity assay. The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids. The total antioxidant capacity of the plant extracts was measured spectrophotometrically at 695 nm and is based on the reduction of Mo (VI)–Mo (V) by the antioxidants with subsequent formation of a green phosphate/Mo (V) complex at acid pH (Brighente et al 2007). As illustrated in Figures 1 & 2, plant extracts with high hydrophilic antioxidant contents consistently exhibited relatively high lipophilic antioxidant contents as indicated by their AAE and BHTE values, respectively.

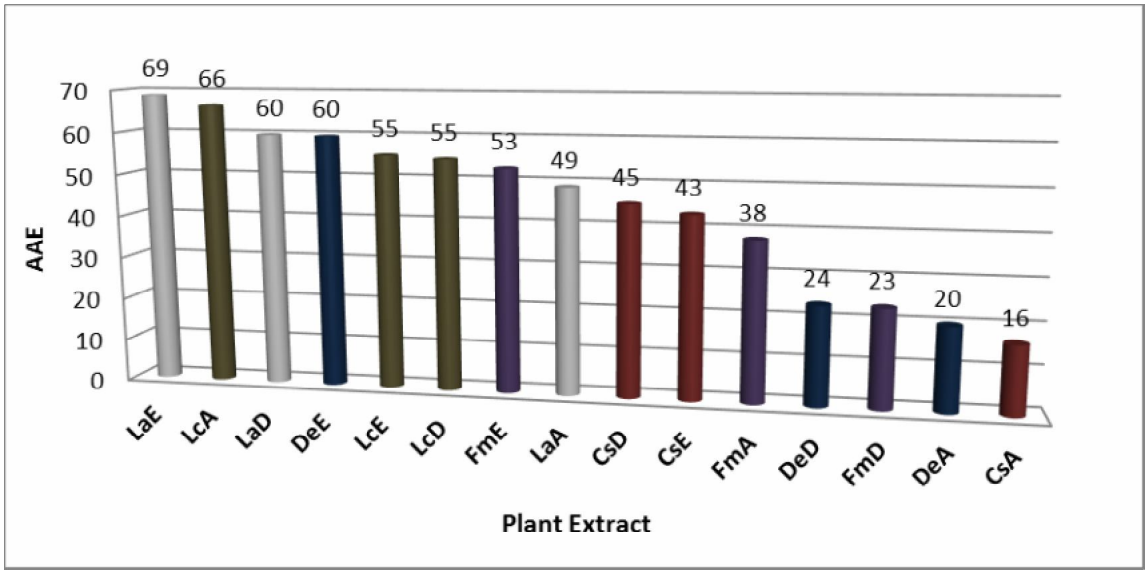

Figure 1. Total antioxidant capacities of the leaf extracts at 200-ppm concentration expressed as ascorbic acid equivalents (AAE) arranged in decreasing order.

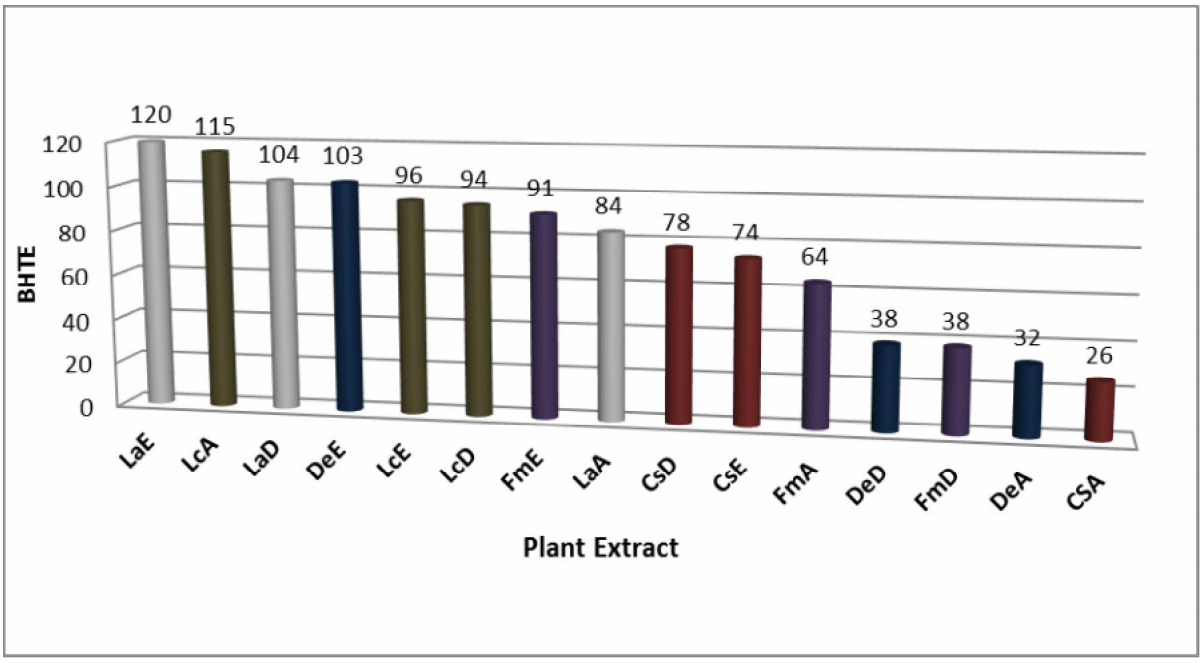

Figure 2. Total antioxidant capacities of the leaf extracts at 200-ppm concentration expressed as butylated hydroxytoluene equivalents (BHTE) arranged in decreasing order.

In general, all plant extracts have higher BHTE values than AAE values indicating that they possess more lipophilic antioxidant than hydrophilic antioxidants. Moreover, the top five plant extracts in terms of total antioxidant capacities expressed both as AAE and BHTE values are the ethanol extract of *L. aculeata*, LaE (69 AAE , 120 BHTE), the EtOH:H_2O extract of *L. capitellata*, LcA (66 AAE, 115 BHTE), the decoction of *L. aculeata*, LaD (60 AAE, 104 BHTE), the ethanol extract of *D. elliptica*, DeE (60 AAE, 103 BHTE) and the ethanol extract of L. capitellata, LcE (55 AAE, 96 BHTE).

Total phenolics content assay. It has long been recognized that phenolic compounds have strong ability to scavenge radicals, thereby protecting cells against the detrimental effects of reactive oxygen species (ROS) (Hogan et al 2009). The plant extracts were subjected to determination of the total phenolics content measured spectrophotometrically and expressed as Gallic Acid Equivalence (GAE, mg gallic acid/g sample). The antioxidant properties of plant extracts can be attributed to their phenolic compounds (Soong & Barlow 2004; Ismail et al 2004; Song et al 2010; de Oliveira et al 2012). Among the plant extracts tested, the top five highest values were shown by the decoction of *L. aculeata* (LaD, 422.76 GAE), the EtOH:H_2O extract of *L. capitellata* (LcA, 335.62 GAE), the decoction of *L. capitellata* (LcD, 278.00 GAE), the ethanol extract of *L. aculeata* (233.71 GAE) and the EtOH:H_2O extract of *F. minahassea* (FmA, 215.62 GAE). The presence of phenolic compounds is reflected by the type of plant extracts having the highest GAE values; the polar extracts contain more of these compounds since such compounds are also relatively polar. Plant leaf extracts having more phenolics content is generally believed to show good antioxidant activity (Brighente et al 2007). It can be stated that phenolics content of the plant may be a good indicator of its antioxidant capacity (Chanda & Dave 2009).

Conclusions. Linear correlations between the DPPH radical scavenging capacity, total phenolic content and the total antioxidant activity of the studied five plant leaf extracts emphasize that these *in vitro* assays are convenient and reliable for the determination of the antioxidant profile of plant leaf extracts. The quantification of phenols and total antioxidant activity was based on the standard curve generated by the use of GAE (R^2 = 0.9939), BHTE with R^2 = 0.9999 and AAE with R^2 = 1, respectively. Among the five plants of interest, the highly ranked antioxidant profiles of plant species *L. capitellata* and *L. aculeata* were noteworthy. The antioxidant results obtained for the remarkable plant *L. aculeata* may be a significant factor for its folkloric uses as poulticing and its aid for fever (http://www.asianplant.net/Vitaceae/Leea_indica.htm). A recent study has established the presence of terpenoids, cardiac glycosides and flavonoids in the leaves of *L. aculeata* (Lagunay & Uy 2015) which could have contributed to these significant antioxidant properties. The antioxidant activity result obtained for *L. capitellata* is in agreement with the studies conducted by Ling (2008) wherein leaf extracts were evaluated to have high total phenolics content and antioxidant activity values.

In vitro assays can only rank antioxidant activities for their particular reaction systems and their relevance to *in vivo* health protective activities is uncertain (Niki 2011; Apak et al 2013). Thus, it is recommended to include at least one *in vivo* assay that has biological relevance. Further purification and isolation of the noteworthy plants in terms of antioxidant activities specifically the *L. capitellata* and *L. aculeata* is also highly recommended.

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