

PHYTOCONSTITUENTS AND *IN VITRO* ANTI-OXIDANT ACTIVITY OF SELECTED INDIGENOUS VEGETABLES IN THE ILOCOS

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ABSTRACT

Various indigenous vegetables abound in Ilocos Norte but are underutilized. Ethnobotanical data present some of their medicinal benefits but these data are not supported by scientific evidence. We identified the secondary metabolites present and evaluated the anti-oxidant property using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay of ethanolic extracts derived from *Brousonetia luzonica*, *Momordica cochinchinensis*, *Telosma procumbens*, *Mollugo verticillata* and *Schismatoglottis* sp.

Qualitative analysis showed that the edible parts of the five species contain coumarins, flavonoids, phenols, steroids, terpenoids, tannins and cardiac glycosides. All species demonstrated free radical scavenging activities with *T. procumbens* showing the greatest activity. Results suggest that the five indigenous vegetables are healthy foods and could be a possible source of nutraceutical products.

Keywords: *ant-oxidant, free radical scavenging activity, indigenous vegetables, phytochemical*

INTRODUCTION

Indigenous vegetables (IV) are vital components of a nutritious diet in the rural communities of Ilocos Norte, in northwestern Luzon, Philippines. A total of 33 species, which are traditionally used as food and for the treatment for certain diseases among Ilocanos and indigenous groups have been documented in the province (Antonio *et al.*, 2011). These species grow in the upland and remote areas, but some are domesticated in home gardens. Most popular and utilized among these are *Brousonetia luzonica* (himbabao), *Telosma procumbens* (kapaskapas), *Mollugo verticillata* (papait), *Schismatoglottis* sp. (bilagot) and *Momordica cochinchinensis* (sugudsugud). The edible parts of the plants, such as flowers, tender leaves or leaftops and fruits are prepared as salad, sautéed, or viand mixed with other vegetables.

Aside from the use of the plants as food, ethnomedicinal testimonies reveal the importance of *M. verticillata* as anti-anemia and anti-hyperglycemia. Meanwhile, *Schismatoglottis* sp. leaves are crushed and used to revive a fainting person (Antonio, Agustin, and Badar, 2016).

Like other vegetable species, IVs are loaded with vitamins, minerals and phytochemicals, which recently marked an essential role in disease prevention through their anti-oxidant properties along with other biological activities (Bumanglag *et al.*, 2011; Saxena, Nema, Singh, and Gupta, 2013). In *B. luzonica* flower extract, β -sitosterol, a steroid compound, and triterpenoids namely: lupenone, lupeol, betulin aldehyde fatty acid ester (BAFAE) and lupeol fatty acid ester (LFAE) have been reported (Tsai, De Castro-Cruz, Shen, C.C., and Ragasa, *et al.*, 2012). Of these, lupenone and BAFAE have been

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found to exhibit low to moderate inhibitory effects on the growth of several bacteria and fungi species. Another study reported the presence of the phytochemicals alkaloids, flavonoids, glycosides, phenols, steroids, tannins and terpenoids in, and similar low antibacterial activity of, the *B. luzonica* flower extract (Ruma, 2015). On the other hand, *T. procumbens* fruits have been found to contain 18 polyoxypregnane glycosides named telosmosides A1 to A18 (steroidal glycoside), of which A15 is 1000 times sweeter than sucrose (Huan, Ohtani, Kasai, and Yamasaki, 2001), and same part exhibited anti-diabetic activity (Cajuday & Amparado, 2014). Moreover, Ferreira et al (2003) reported that *M. verticillata* contained quercetin and other flavonoid glycoside and triterpenoid glycoside and showed immunomodulatory effects in Bacillus Calmette Guerin (BCG) antigen- and *Mycobacterium tuberculosis* whole antigen-induced mice peritoneal cell cultures. Meanwhile, *M. cochinchinensis*, the most studied among the five IVs, exhibited several bioactivities like renal-protection on alloxan-induced diabetic rats (Phani et al., 2013), blood glucose lowering and analgesic (Akhter et al., 2014). Both immature and mature fruits indicated anti-oxidative and antimicrobial activities, which could be ascribed to the terpenoids, flavonoids and other phenolic compounds detected in them (Kubola & Siriamornpun, 2011; Tinrat et al., 2014; Tinrat, 2014). Several bioactivities were reported on the five target species but only *M. cochinchinensis* was shown to exhibit anti-oxidant activity.

The anti-oxidant activity of plant chemicals, which include beta-carotene, vitamins A, C and E, lutein, lycopene and selenium among others, is much associated with protective action specifically on oxidative stress. Although oxidation is necessary to modulate a variety of defense system and cellular responses, the process produces free radicals and reactive oxygen species (ROS) that are harmful to the host (Yoshikawa & Naito, 2002; Rahman, 2007). The radical species are unstable and easily react with proteins, carbohydrates, lipids and DNA

molecules (Lobo et al., 2010) resulting in cellular and metabolic injury, accelerated aging, cancer, cardio-vascular diseases, neurodegenerative diseases and inflammation, among others (Sahu et al., 2013). The anti-oxidants are capable of inhibiting or slowing the oxidants by scavenging the free radicals through hydrogen or electron donation, metal ion chelation, enzyme inhibition, and peroxide decomposition (Sahu et al., 2013; Lobo et al., 2010). In that way, adding vegetables, which are rich in anti-oxidants, into the diet helps improve the immune system, and safeguard the body from above-mentioned disorders (Liu, 2013).

Despite the health benefits IVs could offer, most are less preferred and unrecognized for their food value (Keatinge et al., 2015) until recently when these are gradually being re-discovered for their potential in addressing micronutrient deficiencies and diseases. In the Ilocos provinces, the five IVs identified are important components of the diet of the Ilocanos and other ethnic groups. However, there is limited scientific evidence, particularly on the nutritive and non-nutritive components of the vegetables and bioactivities derived from them, which support their utilization for food and health. In this study, we determined the phytoconstituents and evaluated the anti-oxidant activity of the five IVs. We specifically tested the edible parts: male inflorescence in *B. luzonica*, young fruits in *T. procumbens*, tops in *M. verticillata*, stem and leaves in *Schismatoglottis* and young fruits in *M. cochinchinensis*. Research results can provide the scientific basis for their traditional use as vegetables by local communities and developing the plants as food products and/or nutraceutical products.

Conceptual Framework

The project was carried out on the premise of the conceptual framework presented in Fig. 1. The Province of Ilocos Norte (specific location?) has a wealth of indigenous vegetables and traditional edible plant species that continually sustain the food

requirements of many of its upland and remote communities, and are traditionally used to cure certain diseases or ailments. Indigenous vegetables are good sources of vitamins, minerals and phytochemicals which provide medicinal properties such as anti-diabetic, anticarcinogenic, hypolipidemic, antibacterial and anti-oxidant, among others (Sahu, Kar and Routray, 2013).

However, little has been known of the nutritive and non-nutritive or phytochemical composition of local species. Moreover, there has been no analysis done on these plant materials grown and sampled in the province. A systematic analysis of these local species is essential since phytochemical composition is affected by biophysical factors such as variety, growing condition, season, and cultural management, among others.

The study investigated five IV species and their phytochemical components and anti-oxidant activity measured in terms of their DPPH free radical scavenging action. Results formed scientific support to the use of these species as health-promoting food and as potential sources of antioxidants.

METHODOLOGY

Collection and Authentication of Samples

Fresh *B. luzonica* male inflorescences, *Schismatoglottis* sp. stem and

leaves, *M. cochinchinesis* young fruits and *T. procumbens* young fruits were collected from the plants grown and maintained at the Indigenous Food Plants Botanical Garden of the Mariano Marcos State University (MMSU), City of Batac, Ilocos Norte during the months of February, April and September 2016, respectively. The *M. verticillata* leaftops were gathered from a dried pond in the area in February 2016. The plant materials collected were at their developmental stages preferred for consumption as vegetables in the locality. The unwanted parts were removed and the disease-free inflorescence, fruits and leaves were selected.

Herbarium specimens of each vegetable species were prepared and authenticated by Dr. Wilfredo Vendivil, Chief/Curator II at the Botanical Division, Philippine National Museum.

Preparation of Extracts

The plant materials were thoroughly washed and oven dried (Memmert Model 800, Germany) at 50°C. Dried samples were ground using the sample mill (Foss Cyclotec 1093, Sweden). The extracts were prepared based on the procedures of Siddiqui *et al.* (2009) and Guevarra *et al.* (2005) with slight modifications on the time of soaking. Powdered samples were immersed in 80% ethanol (Labscan,

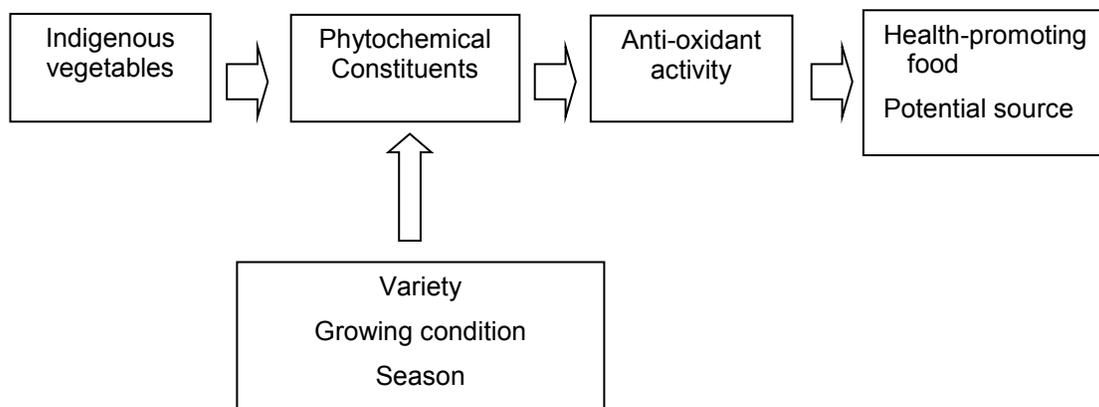


Fig. 1. Several biophysical factors affect the phytoconstituents and anti-oxidant activity of the five indigenous vegetable species from Ilocos Norte

Thailand) at 1:6 w/v ratio and heated on water bath (JSR Model JSWB-22T, Korea) for 1 hour at 50°C. The maceration of the extracts lasted for 72 hours and filtered with Whatman No. 42. Each filtrate concentrated at 50°C on water bath. The crude extracts obtained were stored in jars and kept at -20°C freezer (EVERmed Model LF140W, Italy) until used for analysis.

Phytochemical Screening

Qualitative phytochemical screening was carried out for the usual secondary metabolites adopting the procedures of Tiwari *et al.* (2011) and Himesh *et al.* (2011). The screening was performed for alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, anthraquinones, cardiac glycosides, phenols and coumarins. The color intensity and/or precipitate formed served as bases for the presence or absence of the phytochemical group as required by the test reactions.

DPPH Free Radical Scavenging Assay

The radical scavenging method using 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, USA) is a simple and the most utilized assay to estimate anti-oxidant activities *in vitro* (Molyneux, 2004; and Kedare and Singh, 2011). Hence, the crude extracts were evaluated using the assay following the protocol of Marinova and Batchvarov (2011) with slight modifications on the standard and concentrations used as a result of the optimization process done.

The extracts and gallic acid (Sigma-Aldrich, USA) were dissolved in ethanol at different concentrations (500, 350, 250, 200, 150, 100 and 50 µg/ml). Subsequently, 1.5 ml of each sample was reacted with an equal volume of 0.06 mM DPPH prepared in ethanol. The DPPH, in its oxidized form, is deep violet but turns yellow when reduced through electron donation by the antioxidant compounds from the samples including the standard used.

The control was prepared by mixing the same volume of ethanol and DPPH. Each solution was shaken vigorously and incubated in a dark room for 30 minutes. Absorbance was measured at 517 nm through UV-Vis Spectrophotometer (Labomed Spectro UV-2550, USA). Lower absorbance reading indicates higher activity. The radical scavenging activity (RSA) was

$$\text{RSA (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Extract}}{\text{Absorbance of Control}} \times 100$$

computed using the following equation:

The half maximal inhibitory concentration (IC₅₀) indicating the needed amount of sample to scavenge or inhibit the action of DPPH by 50% was determined using the linear regression of dose inhibition curve plot between concentration of the extracts and radical scavenging activity (MasterPlex Reader Fit 2010 free trial). Low IC₅₀ values would indicate high anti-oxidant activity.

Statistical Analysis

All experiments were done in triplicate. Data in DPPH assay were shown as mean±SD and analyzed using Analysis of Variance in Completely Randomized Design (CRD) in STAR 2.0.1 (IRRI, 2013).

RESULTS AND DISCUSSION

Phytoconstituents of the Indigenous Vegetables

Natural products have been the single most productive source of bioactive compounds for development of drugs. Thus, screening for the phytoconstituents was done on the five IVs which revealed the presence of flavonoid, phenol, steroid, terpenoid, saponin, tannin, cardiac glycoside and coumarin (Table 1).

Among the five species, *B. luzonica* had the most phytochemicals with eight kinds, followed by *T. procumbens* (seven kinds); while *M. cochinchinensis* had the least (five kinds). The male inflorescence from *B. luzonica* contains all secondary metabolites analyzed except alkaloid and anthraquinone. This confirmed the results of other investigations (Ruma, 2015; and Tsai *et al.*, 2012), but the presence of saponin and the absence of alkaloid in the samples did not agree with findings of Ruma (2015). Similarly, *B. papyrifera*, *B. kazinoki* and *B. zeylanica* yielded about 100 new and common phytoconstituents (Kinghorn, 2003) in various forms of alkaloids, phenols, flavonoids, coumarins, glycosides, lignans and diterpenes. These were distributed in the roots, bark, leaves, flowers and fruits where according to the review done by Wang *et al.* (2012), many of these constituents demonstrated biological activities such as anti-oxidant, anti-microbial, anti-inflammatory and anti-diabetic, among others.

The young fruits of *T. procumbens* fruits had flavonoid, phenol, steroid, terpenoid, tannin, cardiac glycoside and coumarin, showing it to be more promising than its edible flowers having only anthraquinones, flavonoids, glycosides and

steroids (Ruma, 2015). The detected steroids in the extract could also constitute the telosmosides elucidated by Huan and colleagues (2001). Congeners *T. pallida* and *T. africanum* possessed the same group of compounds in addition to combined anthraquinones, saponin and alkaloid (Sharma *et al.*, 2014; Adediwura & Ayotunde, 2012). However, the extracts analyzed by the latter were from the leaves and stems.

The phytochemicals found in *M. verticillata* leafy tops and *Schismatoglottis* sp. stem and leaves extracts comprised six groups. The presence of flavonoids and triterpenoid in the *M. verticillata* extract verified the study of Ferreira *et al.* (2003). Although only few reports show its phytochemistry, *Mollugo* species *M. oppositifolia*, *M. pentaphylla* and *M. nudicaulis* had similar phytoconstituents as *M. verticillata*. Alkaloid, saponin and tannin were undetected in this study. The mentioned plants have indicated medicinal properties such as anti-oxidant (Rajamanikadan *et al.*, 2011; Jagatheesh *et al.*, 2011), anti-microbial, anti-inflammatory, anti-tumor (Jagatheesh *et al.*, 2011); antihyperglycemic and anti-diabetic (Gopinathan & Subha, 2014). On the other

Table 1. Phytochemical constituents of selected indigenous vegetables in Ilocos Norte

PLANT SPECIES	PHYTOCHEMICALS										
	Alk	Fla	Ant	Phe	Ste	Ter	Sap	Tan	Car	Cou	
<i>Broussonetia luzonica</i>	-	+	-	+	+	+	+	+	+	+	
<i>Mollugo verticillata</i>	-	+	-	+	+	+	-	-	+	+	
<i>Momordica cochinchinensis</i>	-	-	-	+	+	+	-	-	+	+	
<i>Schismatoglottis</i> sp.	-	+	-	+	+	+	-	-	+	+	
<i>Telosma procumbens</i>	-	+	-	+	+	+	-	+	+	+	

Legend: (+) Present; (-) Absent; Alk – alkaloid; Fla – flavonoid; Ant – anthraquinone; Phe – phenol; Ste – steroid; Ter – terpenoid; Sap – saponin; Tan- tannin; Car – cardiac glycoside; Cou - coumarin

hand, like the *Schismatoglottis* sp. sample, *S. ahmadii* leaves and stem had phenol and flavonoid with anti-oxidant activities (Wong *et al.*, 2013).

Furthermore, phenol, steroid, terpenoid, cardiac glycoside and coumarin were found in the young fruits of *M. cochinchinensis*. The crude extract was negative for flavonoid, which did not corroborate the findings of Kubola and Siriamornpun (2011) in which the flavonoids myricetin, luteolin, quercetin, apigenin, rutin and kaempferol were identified. *M. cochinchinensis* possessed some secondary metabolites similar to *M. charantia*. However, *M. cochinchinensis* seemed less explored than *M. charantia* in which about 228 phytochemicals were already documented from all parts of the latter. Majority of *Momordica* plants' biologically active chemicals were phenolics, flavonoids, carotenoids, cucurbitane triterpenoids and phytosterols (Nagarani *et al.*, 2014). Moreover, alkaloids and saponins occurred in the fruits of *M. charantia* (Singh *et al.*, 2012), *M. dioicia* (Talukdar and Hossain, 2014), and *M. balsamina* (Faujdar *et al.*, 2013).

Anti-oxidant Activity

Anti-oxidants are richly found in fruits and vegetables and protect the human body from oxidative stresses leading to cellular damage, certain diseases and disorders (Mandal, 2015). The anti-oxidant activity of the five vegetable species was measured using their scavenging activity on the free radical DPPH. The extracts from the five species exhibited significant differences ($p < 0.05$) in all the concentration levels tested (Table 2). Among the species, *T. procumbens* fruits had the highest efficacy (93.7±0.09 % at 500 µg/ml) and the lowest IC₅₀ (39.80 µg/ml). Efficacy is the maximal effect of a compound or drug and is measured here in terms of percent RSA. Meanwhile, the IC₅₀ measures the potency and is an expression of the activity of a drug or compound in terms of the concentration or amount required to produce a defined effect (Waldman, 2002). So, the lower the IC₅₀

value, the lesser the concentration of a drug or compound required to produce 50% of maximum effect, and the higher the potency. Thus, *T. procumbens* fruit extract both exhibited the highest efficacy and potency as antioxidant.

In a study done on another *Telosma* species, *T. minor*, the ethanol and water extracts of flowers demonstrated DPPH scavenging activities which were 42.9% and 54.5% lower, respectively, than *T. procumbens* fruits at 100 µg/ml (Sawatpipat *et al.*, 2013). Meanwhile, *T. pallida* leaves extracted using different solvents had IC₅₀ values of 253±1.02 µg/ml (methanol), 323±0.49 µg/ml (ethyl acetate) and 481±0.70 µg/ml (water) while the stem had 158±0.48 µg/ml (methanol), 202±0.42 µg/ml (ethyl acetate) and 279±0.92 µg/ml (water) (Sharma *et al.*, 2014). These IC₅₀ values are 118 to 441 µg/ml greater than the IC₅₀ of *T. procumbens*. This indicates that *T. procumbens* fruit is a more potent anti-oxidant than *T. pallida* leaves and stem.

Following the efficacy of *T. procumbens* are *B. luzonica* (89.95±0.15%), *Schismatoglottis* sp. (81.9±0.09%) and *M. cochinchinensis* (73.16±0.09%). A similar trend was observed on the potency of *B. luzonica* (IC₅₀ 79.34 µg/ml), and *Schismatoglottis* sp. (IC₅₀ 100.76 µg/ml) but not on *M. cochinchinensis* (IC₅₀ 3,062.05 µg/ml) (Table 2). *B. luzonica* inflorescence had higher DPPH activity (89.95%±0.15%) than the fruits and flowers of its relative *B. papyrifera* (87.17±0.18% and 62.88%, respectively, at 5000 µg/ml) (Sun *et al.*, 2012; and Sun *et al.*, 2011). Meanwhile, *M. verticillata* leaf tops had the lowest anti-oxidant activity (51.2±0.09%). In comparison with other *Mollugo* species, *M. pentaphylla* and *M. cerviana* are more promising anti-oxidants than *M. verticillata*. *M. cerviana* significantly inhibited free radicals by 84.12±1.06% at 400 µg/ml (Valarmathi *et al.*, 2015). In addition, *M. pentaphylla* and *M. cerviana* only require IC₅₀ levels of 16 µg/ml (Priya *et al.*, 2017) and 99 µg/ml (Napagoda *et al.*, 2016), respectively, as compared to *M. verticillata* which requires 405.86 µg/ml.

Table 2. The DPPH radical scavenging activity (%) of the ethanolic extract of the five indigenous vegetable species

SPECIES	CONCENTRATION ($\mu\text{g/ml}$)							IC ₅₀ ($\mu\text{g/ml}$)
	50	100	150	200	250	350	500	
Galic Acid	94.6±0.07 a	95.2±0.10 a	95.6±0.07 a	95.7±0.08 a	95.8±0.14 a	95.9±0.08 a	96.3±0.04 a	15.79
<i>B. luzonica</i>	28.9±1.74 e	67.1±1.89 b	86.9±0.52 b	89.0±0.26 b	89.4±0.30 b	89.8±0.26 b	89.9±0.15 c	79.34
<i>M. verticillata</i>	45.1±0.09 d	45.7±0.09 e	46.5±0.09 f	46.7±0.09 f	48.3±0.109 f	49.4±0.09 f	51.2±0.09 f	405.86
<i>M. cochinchinensis</i>	47.3±0.31 c	52.2±0.15 d	54.7±0.09 e	58.1±0.15 e	60.7±0.16 e	65.8±0.40 e	73.2±0.09 e	3,062.05
<i>Schismatoglottis</i> sp.	49.1±0.26 bc	54.6±0.00 c	59.3±0.00 d	63.2±0.35 d	67.5±0.15 d	74.3±0.15 d	81.9±0.09 d	100.76
<i>T. procumbens</i>	50.1±0.09 b	56.6±0.00 c	62.4±0.09 c	68.3±0.09 c	73.8±0.09 c	84.6±0.15 c	93.7±0.09 b	39.80
CV (%)	1.40	1.26	0.3298	0.2847	0.2339	0.2845	0.1172	

* - Significant at 0.05 level, CV - coefficient of variation, \pm - standard deviation
 In a column, means with the same letter are not significantly different at LSD 0.05

Nevertheless, *M. verticillata* is more potent than *M. nudicaulis* (IC₅₀ 1,350 µg/ml) (Rajamanikandan *et al.*, 2011) including *M. cochinchinensis* (IC₅₀ 3,062.05 µg/ml).

The low potency observed in the young fruits of *M. cochinchinensis* analyzed in this study corroborated the findings of Kubola and co-workers (2011). The latter reported IC₅₀ of 2,560±0.09 µg/ml in immature green peel and 2,350±0.01µg/ml in pulp, which were lower than the IC₅₀ of medium ripe (yellow) and fully ripe (red) *M. cochinchinensis* fruits. Nevertheless, the *M. cochinchinensis* ethanol fruit extract analyzed in this study gave 73.16±0.09% free radical scavenging activity at 500 µg/ml in contrast to *M. charantia* ethyl acetate and ethanol fruit extracts which gave 73.75% and 77.65%, respectively, at a higher concentration of 800 µg/ml. The latter, however, were more potent DPPH radical scavengers for having IC₅₀ of 157 µg/ml and 112 µg/ml, respectively (Talukder *et al.*, 2013). Another *Momordica* species, *M. cymbalaria*, gave 74.9% inhibition at 100 ug/ml and IC₅₀ of 42 µg/ml

from its aqueous fruit extract (Rajasekhar *et al.*, 2010).

The standard, gallic acid, had the highest antioxidant activity in all concentrations as manifested in higher scavenging activity than the crude extracts of the five plant species. The five plant species likewise showed varying antioxidant activities (p>0.05). Among the plants, *T. procumbens* showed the highest antioxidant activity at 50 ug/ml and 500 ug/ml levels. But starting at 100 ug/ml to 350 µg/ml, *B. luzonica* consistently had the highest antioxidant activity. From an initial low activity at 50 µg/ml, *B. luzonica* activity significantly increased as the concentration is increased (Fig 2). Moreover, the vegetable crude extracts except for *M. verticillata* have reached about 50% antioxidant activity at 100 µg/ml. Moreover, the trend for all the species followed a concentration-dependent activity (Fig 2). Anti-oxidant activity increases as the concentration of the extracts used is increased. The detection of terpenoids and phenols which include flavonoid, tannin and

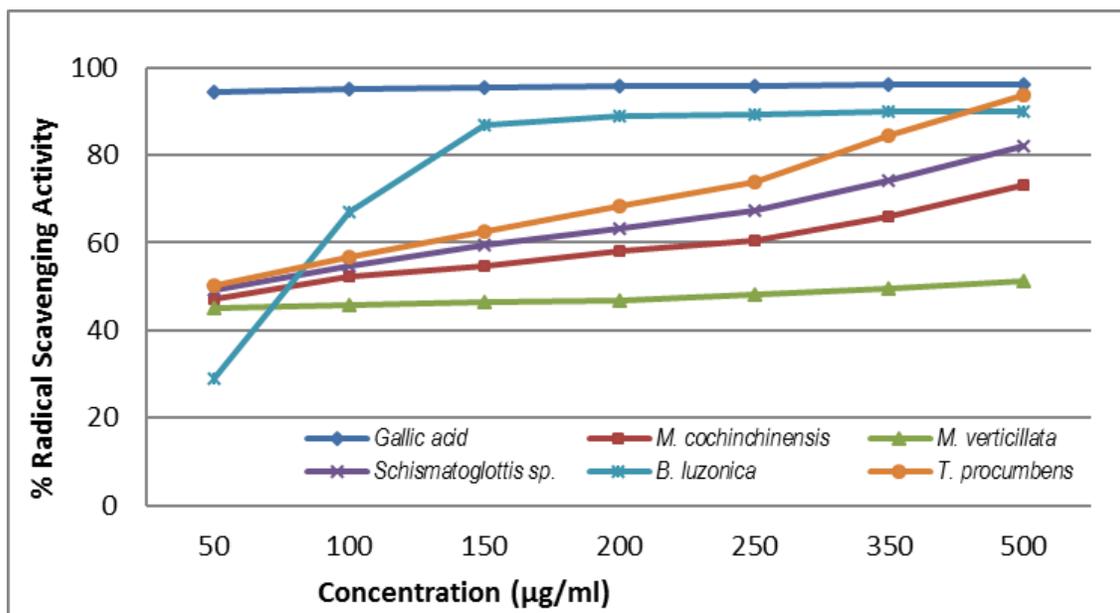


Fig 2. The radical scavenging activity (%) of the five indigenous vegetable species showing the concentration-dependent trend

coumarin makes the identified indigenous vegetables promising as anti-oxidants. The essential oils in plants containing monoterpenes (eg. limonene, perillyl alcohol, carvone), sesquiterpenes (e.g. alpha and beta-caryophyllene) and diterpenes (e.g. vitamin A); including the plant pigments, tetraterpenes or carotenoids (e.g. β -carotene, lutein and violaxanthin) are known to possess antioxidant properties *in vitro* and *in vivo*. The anti-oxidant capacity of monoterpenes, sesquiterpenes and diterpenes especially those without phenolic is due to the fast termination of the chain reaction by chain carrying HOO⁻ radicals that react rapidly with linoleylperoxyl radicals. Meanwhile, the carotenoids inhibit oxidation by quenching singlet oxygen and peroxy radicals through electron donation, hydrogen abstraction or radical addition. These compounds work in synergy with other antioxidants such as rutin, a flavonoid and vitamin E, α -tocopherol (Grabmann, 2005)

Similarly, the phenolic groups are noteworthy radical species scavengers, metal ion chelators and defense system regulators. These have been proven to have stronger properties than the earlier reported antioxidants (e.g. carotenoids, vitamin C, and vitamin E). Phenolics can easily donate an electron or hydrogen atom and delocalize a single electron because of their phenolic hydroxyl groups and extended conjugated aromatic system (Dai & Mumper, 2010). Hence, the difference on the anti-oxidant activities of the plants could be ascribed to their phytochemicals contents. The presence of the three phenols (flavonoid, tannin and coumarin) in both *T. procumbens* and *B. luzonica* account for their high anti-oxidant activities. However, elucidation of the chemical constituents is necessary to further establish the antioxidant properties of these indigenous vegetables.

CONCLUSIONS AND RECOMMENDATIONS

The five indigenous vegetables are rich in phytochemicals and showed anti-oxidant activities, which support their

traditional use as food. The potency of the species is in the decreasing order: *T. procumbens* > *B. luzonica* > *Schismatoglottis* sp. > *M. verticillata* > and *M. cochinchinensis*. The elucidation of the chemical fingerprint along with the nutritive components of the vegetable species is necessary to validate the ethnomedicinal uses, determine the anti-oxidant compounds and identify other therapeutic applications. Other biological screening is also important to establish the medicinal properties of above vegetable species.

LITERATURE CITED

- Antonio, M.A., Utrera, R.T., Agustin, E.O., Jamias, D.L., Badar, A.J. and Pascua, M.E.** (2011). Survey and characterization of indigenous food plants in Ilocos Norte, Philippines. *SEARCA Agriculture and Development Discussion Paper Series*. SEARCA, Los Banos, Laguna.
- Antonio, M.A., Agustin, E.O. and Badar, A.J.** (2016). *Catalog of indigenous food plants in Ilocos Norte*. City of Batac, Ilocos Norte, Philippines, Mariano Marcos State University. ISBN 978-971-790-131-2.: 82 p.
- Aoki, H., Kieu, N.T.M., Kuze, N., Tomisaka, K. and Chuyen, N.V.** (2014). Carotenoid pigments in gac fruit (*Momordica cochinchinensis* Spreng). *Bioscience, Biotechnology, and Biochemistry*. 66:11, 2479-2482. ISSN 1347-6947. DOI: 10.1271/bbb66.2479. Retrieved from www.tandfonline.com/loi/tbbb20 on July 15, 2016.
- Bumanglag, M.M., Devanadera, M.A.R., Pua, M.D., Salen, C.S., Reyes, A.R. and Capanzana, M.V.** (2011). Lutong FNRI: Mga katutubong gulay. *Food and Nutrition Research Institute*. ISBN: 978-971-91051-9-0. Retrieved from <http://www.fnri.dost.gov.ph/index.php/90-news/103-lutong-fnri-mga-katutubong-gulay>.

- Dai, J. and Mumper, J.** (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15, 7313-7352. ISSN 1420-3049. DOI:10.3390/molecules15107313. Retrieved from www.mdpi.com/journal/molecules on August 15, 2016.
- Doughari, J.H.** (2012). Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents, phytochemicals – A global perspective of their role in nutrition and health, Dr. Venketeshewer Rao (Ed.). ISBN 978-953-51-0296-0. Retrieved from www.intech.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-and-health/phytochemicals-extraction-methods-basic-structures-and-mode-of-action-as-potential-chemotherapeutic.
- Doughari, J.H.; Human, I.S., Bennade, S. and Andndakidemi, P.A.** (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*, 3:11, 839-848. ISSN 1996-0875. Retrieved from www.academicjournals.org/jmpr on August 15, 2016.
- Ferreira, A.P., Soares, G.L.G., Salgado, C.A., Goncalves, L.S., Teixeira, F.M., Teixeira, H.C. and Kaplan, M.A.C.** (2003). Immunomodulatory activity of *Mollugo verticillata* L. *Phytomedicine*, 10, 154-158. Retrieved from www.urbanfischer.de/journals/phytomed.
- Grabmann, J.** (2005). Terpenoids as plant antioxidants. *Vitam Horm.* 72, 505-535. DOI: 10.1016/S0083-6729(05)72015-X. Retrieved from www.researchgate.net/publication/7285715_Terpenoids_as_Plant_Antioxidants on July 15, 2016.
- Himesh, S., Sharma, S., Patel, S.S., Singhai, M.K., Mishra, K. and Singhai, A.K.** (2011). *International Research Journal of Pharmacy*, 2(5), 242-246. Retrieved from https://www.researchgate.net/publication/285321018_Preliminary_Phytochemical_Screening_and_HPLC_Analysis_of_Flavonoid_From_Methanolic_Extract_of_Leaves_of_Anonna_squamosa.
- Huan, V.D., Ohtani, K., Kasai, R. and Yamasaki, K.** (2001). Sweet pregrane glycosides from *Telosma procumbens*. *Chemical and Pharmaceutical Bulletin*, 49 (4), 453-460. DOI: 10.1248/cpb.49.453.
- Keatinge, J.D.H., Wang, J.-F., Dinssa, F.F., Ebert, A.W., d'a. Hughes, J., Stoilova, T., Nenguwo, N. Dhillon. . . and Ravishankar, M.** (2015). Indigenous vegetables worldwide: their importance and future development. *Acta Horticulturae*. 1102. ISHS 2015. DOI 10.17660/actahortic.2015.1102.1.
- Kedare, S.B. and Singh, R.P.** (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*. 48:4, 412-422. DOI 10.1007/s13197-011-0251-1. Retrieved from www.ncbi.nlm.nih.gov/pmc/articles/PMC3551182/ on April 28, 2016.
- Kubola, J. and Siriamornpun, S.** (2011). Phytochemicals and anti-oxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). *Food Chemistry*, 127, 1138-1145. DOI:10.1016/j.foodchem.2011.01.115.
- Liu, R.H.** (2013). Health-promoting components of fruits and vegetables in the diet. *American Society for Nutrition. Advances in Nutrition*, 4, 384-392. DOI:10.3945/an.112.003517.
- Lobo, V., Patil, A., Phatak, A. and Chandra, N.** (2010). Free radicals, antioxidants and functional foods: impact on human health. *Pharmacognosy Review*, 4(8). DOI:10.4103/0973-7847.70902.

- Mai, H.C., V. Truong, B. Haut, and F. Debaste.** (2013). Impact of limited drying on *Momordica cochinchinensis* S. Carotenoids content and antioxidant activity. *Journal of Food Engineering* 118, 358–364. Retrieved from www.elsevier.com/locate/jfoodeng July 15, 2016.
- Mandal, A.** (2015). What are antioxidants? Retrieved from www.news-medical.net/health/What-is-Oxidative-Stress.aspx on October 21, 2015.
- Marinova, G. and Batchvarov, V.** (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgarian Journal of Agriculture Science*. 17:1, 11-24. Retrieved from www.agrojournal.org/17/01-02-11.pdf on October 21, 2015.
- Molyneux, P.** (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal Science and Technology*. 26:2, 211-219.
- Prassas, I. and Diamandis, E.P.** (2008). Novel therapeutic applications of cardiac glycosides. (2008). *Nature Reviews Drug Discovery*. 7, 926-937. DOI:10.1038/nrd2682. Retrieved from www.nature.com/reviews/drugdisc on March 17, 2017.
- Rahman, K.** (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging*, 2(2), 219-236. Retrieved on <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2684512/>.
- Rohini, K. and Srikumar, P.S.** (2014). Therapeutic role of coumarins and coumarin-related compounds. *Journal of Thermodynamics and Catalysis*. 5:130. ISSN: 2157-7544. Retrieved from DOI:10.4172/2157-7544.1000130 on March 17, 2017.
- Ruma, O.C.** (2015). Antimicrobial activity and phytochemical screening of selected indigenous food plants from Isabela, Philippines. *Upland Farm Journal*, 23 No. 1. Retrieved from <https://ufj.ifsu.edu.ph/antimicrobial-activity-and-phytochemical-screening-of-selected-indigenous-food-plants-from-isabela-philippines/>.
- Sahu, R.K., Kar, M. and Routray, R.** (2013). DPPH Free Radical Scavenging Activity of Some Leafy Vegetables used by Tribals of Odisha, India. *Journal of Medicinal Plants Studies* 1(4), 21-27. Retrieved from www.plantsjournal.com on July 15, 2016.
- Saxena, M., Saxena, J., Nema, R., Singh, D., and Gupta, A.** (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry*, 1 No. 6. Retrieved from <http://www.phytojournal.com/archives/2013/vol1issue6/PartA/26.pdf>.
- Tinrat, S.** (2014). Comparison of antioxidant and antimicrobial activities of unripe and ripe fruit extracts of *Momordica cochinchinensis* Spreng (gac fruit). *International Journal of Pharmacological Science, Review and Research*, 28(1) No. 14, 75-82. Retrieved from https://www.researchgate.net/publication/287308232_Comparison_of_antioxidant_and_antimicrobial_activities_of_unripe_and_ripe_fruit_extract_of_momordica_cochinchinensis_spreng_gac_fruit
- Tiwari, P., Bimlesh, K., Mandeep, K., Gurpreet, K. and Kaur, H.** (2011). Phytochemical screening and extraction: A review. *International Pharmaceutica Scientia*, 1(1), 98-106. <https://www.researchgate.net/file.PostFileLoader.html?id=52ecff43cf57d71b748b45c9&assetKey=AS%3A272427580362754%401441963204700>.
- Tran, X., Parks, S.E., Roach, P.D., Golding, J.B. and Nguyen, M.H.** (2015). Effects of maturity on physicochemical properties

of Gac fruit (*Momordica cochinchinensis* Spreng.). *Food Science and Nutrition*. 4:2,305–314. ISSN 2048-7177. Retrieved from DOI:10.1002/fsn3.291 on July 15, 2016.

Tsai, P., De Castro-Cruz, K.A., Shen, C.C. and Ragasa, C.Y. (2012). Chemical constituents of *Broussonetia luzonica*. *Pharmacognosy Journal*. 4(31). DOI: 10.5530/pj.2012.31.1.

Waldman, S.A. (2002). Does potency predict clinical efficacy? Illustration

through an antihistamine model. *Annals of Allergy Asthma and Immunology*. 89:1, Jul, 7-11; quiz 11-2, 77. Retrieved from DOI:10.1016/S1081-1206(10)61904-7 on July 1, 2017.

Yoshikawa, T. and Naito, Y. (2002). What is oxidative stress? *Journal of Japan Medical Association*, 45(7), 271-276. Retrieved from http://www.med.or.jp/english/pdf/2002_07/271_276.pdf.