World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:9, No:9, 2015

Selected Ethnomedicinal Plants of Northern Surigao Del Sur: Their Antioxidant Activities in Terms of Total Phenolics, ABTS Radical Cation Decolorization Power, and Ferric Reducing Ability

Gemma A. Gruyal

Open Science Index, Bioengineering and Life Sciences Vol:9, No:9, 2015 publications.waset.org/10002103/pdf

***Abstract*—**Plants can contain a wide variety of substances with antioxidative properties which are associated with important health benefits. These positive health effects are of great importance at a time when the environment is laden with many toxic substances. Five selected herbal plants namely, *Mimosa pudica, Phyllanthus niruri, Ceiba pentandra, Eleusine polydactyla* and *Trema amboinensi*, were chosen for the experiment to investigate their total phenolics content and antioxidant activities using ABTS radical cation decolorization power, and ferric reducing antioxidant power. The total phenolic content of each herbal plants ranges from 0.84 to 42.59 mg gallic acid equivalent/g. The antioxidant activity in the ABTS radical cation decolorization power varies from 0.005 to 0.362 mg trolox equivalent/g and the FRAP ranges from 0.30 to 28.42 mg gallic acid equivalent/g. Among the five medicinal plants, *Mimosa pudica* has been an excellent performer in terms of the 3 parameters measured; it is followed by *Phyllanthus niruri*. The 5 herbal plants do not have equivalent antioxidant power. The relative high values for *M. pudica* and *P. niruri* supports the medicinal value of both plants. The total phenolics, ABTS and FRAP correlate strongly with one another.

***Keywords*—**ABTS, FRAP, leaf extracts, phenol.

I. Introduction

OXIDATION process is one of the routs for producing free radicals in food, drugs and even living systems [1]. It is thought that free radicals play an important role in many diseases such as chronic and degenerative disease including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer [2], [3]. The free radicals are formed due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress that cause depletion of immune system antioxidants [4], [5]. Recently, a large number of researches in interest of naturally occurring antioxidants have been conducted due to their considerable multifaceted characteristic seen in their activity of providing enormous scope in correcting imbalance activity particularly treating, preventing diseases and maintaining human health [6].

For thousands of years, nature has been a source of medicinal agents. Plants as medicine have been used for years to provide health coverage for over 80% of the world's population particularly in the developing world [7]. Plants can contain a wide variety of substances with antioxidative properties which are associated to important health benefits when the environment is laden with many toxic substances. Numerous plants have been identified as having potential antioxidant activities and their consumption is recommended [8], [9].

A phenolic compound which is widely distributed in plants has been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic and was also suggested to be a potential iron chelator [10]. In recent year, herbal plants are rapidly becoming popular as a source of an alternative therapy [11].

Mimosa pudica Linn. (Hibi-hibi) is a plant that belongs to family *Fabaceae*. It is a common weed widely distributed in the Philippines in open, moist and open grasslands. Traditionally, it is used for the treatment of traumatic injury, pulmonary tuberculosis, hypertension and diabetes through decoction or infusion [12].

Phyllanthus niruri (Likod) belongs to family *Euphorbiaceae*. It is an herb growing commonly in the roadside as well as in the garden as a weed. Customarily, *P. niruri* is used to treat problems related to the gastrointestinal and genitourinary tracts [13].

Trema amboinensis (Hanagdong) belongs to family *Ulmaceae*. Traditionally, the leaves are used to treat coughs, sore throats, dysenteries and hypertension and the bark is used to make a cough syrup [14].

Ceiba pentandra L. (Gapas) is a plant that belongs to family *Malvaceae* which was previously separated from the family *Bombaceae*. In West Africa, it is generally used in the treatment of diarrhea [15].

Eleusine polydactyla (Busikad) belongs to family *Cyperaceae*. Customarily it is used for cough, fever and muscle aches [16].

The search for novel natural antioxidants from plant origin has increased ever since. In the present study the aim is to determine the total phenolic content and the related total antioxidant potential in five selected ethnomedicinal plants in Surigao del Sur using ABTS radical cation decolorization power and ferric reducing activity power (FRAP).

G.A. Gruyal is with Surigao del Sur State University-Cantilan, Cantilan, Surigao del Sur, Philippines (e-mail: gemma_gruyal@yahoo.com).

Open Science Index, Bioengineering and Life Sciences Vol:9, No:9, 2015 publications.waset.org/10002103/pdf

II. Materials and Methods

A .Plant Material

The samples of *M. pudica* leaf (MPL), *P. niruri* leaf (PNL), *C. pentandra* leaf (CPL), *E. polydactyla* leaf (EPL) and *T. amboinensi* leaf (TAL) were collected during the month of August, 2014 in and around the Northern part of Surigao del Sur, Philippines. These plant materials were botanically authenticated and were deposited in the herbarium at Mindanao University of Science and Technology, Cagayan de Oro City.

B. Plant Preparation

The collected MPL, PNL, CPL, EPL and TAL were sorted and washed with tap water. These plant materials were air-dried thoroughly under shade for 1-2 weeks at room temperature. The dried materials were milled into a fine particle size, place in air tight bottles and stored in the refrigerator at 4^{o}C for subsequent analysis.

C. Chemicals

All the chemicals used for analysis were of analytical grade. Folin-Ciocalteau reagent, gallic acid, quercetin, sodium carbonate (Na_2CO_3), potassium persulfate;2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS); 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (Trolox); potassium persulfate; sodium phosphate; potassium ferricyanide; trichloroacetic acid; Ferric chloride were purchased from Sigma Co. (St. Louis, MO, USA). Other reagents were obtained from Merck Co. (Darmstadt, Germany).

D. Plant Extract Preparation

Twenty (20) grams of each dried grounded plant samples were extracted twice (1500 ml for each sample) with 95% methanol at 20^{o}C for 48 h and concentrated to 100 ml using a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan). The extracts obtained were evaporated under pressure at 50^{o}C to a constant weight. The weight of the filtrate was then transferred to the volumetric flask and raised to 250 ml volume. The extracts were then placed into the storage bottle and placed in the refrigerator at 4^{o}C for succeeding activities.

E. Determination of Plant Extract Yield

Twenty (20) ml of extract solution was pipetted into an aluminum dish (pre-weighed). The dish was placed in an oven to evaporate the methanol at a temperature three degrees (3^{O}) higher than the boiling point of methanol. This was then oven dried for several hours until constant weight of the plant residues were achieved. The yield of each plant extract was calculated by dividing residue by volume of sample (20 ml).

F. Assaying Methods

1. Total Phenolic Contents Determination

The TPC of each methanolic extracts sample were determined according to the Folin-Ciocalteu method [16]. In brief, 300 µL of extract was dispensed into test tube mixed with 1.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 1.2 ml of 7.5% (w/v) Na_2CO_3 solution was added to the mixture followed by the addition of 15 ml of dionized distilled water and mixed thoroughly and allowed to stand for 30 minutes in the dark room at room temperature, after which the absorbance was read at 765 nm. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample and the values are presented as means of triplicate analysis.

2. ABTS Radical-Scavenging Ability

The free radical scavenging activity of *M. pudica* leaf *(MPL), P. niruri* leaf *(PNL), C. pentandra* leaf *(CPL), E. polydactyla* leaf *(EPL)* and *T. amboinensi* leaf *(TAL)* extracts were determined by ABTS (2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical cation with slight modification [17]. The ABTS radical cation (ABTS+) was generated by reacting equal volume of 7 mM ABTS aqueous solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) and the mixture was allowed to stand in the dark for 12-16 hrs at room temperature before use. Prior to use in the assay, the ABTS radical cation solution was diluted with 95% ethanol to an absorbance of about 0.70± 0.02 at 734nm and equilibrated at 30^{o}C. Free radical scavenging activity was assessed by mixing 0.8 ml appropriate dilution of the plant extract was added to 8.0 ml ABTS radical cation solution and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 15 min and the absorbance was measured at 734 nm. All determinations were carried out at three trials. The antioxidant capacity based on the ABTS free radical scavenging ability of the extracts were expressed as mg Trolox equivalents per gram of the dried plant leaves.

3. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing property of the plant extracts was determined using the method described by [18]. Various concentrations of each extract (2.5 ml) were mixed with 2.5 ml of 200mM, pH 6.6 sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The reaction mixture was incubated at 50^{O}C for 20 min followed by addition of 2.5 ml of 10% trichloroacetic acid. The mixture was centrifuged at 650 rpm for 10 min to collect the upper layer of the solution. A volume of 5 ml supernatant was mixed with 5 ml of water and 1 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. The ferric reducing antioxidant activity was then calculated. The higher absorbance of the reaction mixture indicates a higher reducing activity.

G. Statistical Analysis

The data were expressed as the mean ± standard deviation of triplicate separate observations and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 17.

Open Science Index, Bioengineering and Life Sciences Vol:9, No:9, 2015 publications.waset.org/10002103/pdf

III. Results and Discussions

A. Total Phenolic Contents

Table I shows an overview of extraction yields and total phenolic contents of methanolic extracts of *M. pudica, P. niruri, C. pentandra, E. polydactyla and T. amboinensi*

TABLE I
EXTRACTION YIELD AND TOTAL PHENOL CONTENT OF THE FIVE PLANT EXTRACT

Herbal Plant	Extraction Yield (mg/ml)	Total Phenolics Content (mg GAE/g)	SD
M. pudica	18.07	42.59 ±	3.11
P. niruri	9.10	24.01 ±	8.13
C. pentandra	12.97	6.04 ±	0.07
E.polydactyla	5.10	0.84 ±	0.08
T. amboinensi	11.38	9.13 ±	0.27

Results represent the mean ± standard deviation of triplicate readings. Phenolics content are expressed as Gallic acid equivalents (GAE)

There was a wide range of extraction yield and phenolic contents in methanolic plant extracts analyzed as shown in Table I. The values varied from 5.10 to 18.07 mg/ml and 0.84 to 42.59 mg GAE/g respectively. It is known that phenolic compounds derived from plants are well renowned to exhibit antioxidant activity through numerous mechanisms including free radical scavenging lipid peroxidation and chelating of metal ions [19]. Among the five selected ethnomedicinal plants only two methanolic plant extracts had phenolic content > 20 mg GAE/g: M. pudica > P. niruri. The rest of the plants extracts were < 20 mg GAE/g phenolic content. The highest phenolic content (>40 mg GAE/g) was found in M. pudica. According to [20] the antioxidant activity of phenolic compounds this is mainly due to their redox properties which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.

B. ABTS Radical-Scavenging Activity

ABTS radical scavenging assay measured ability to generate a blue/green $ABTS^+$ chromophore via the reaction between ABTS and potassium persulfate. The ABTS radical cation has been often used in the evaluation of antioxidant activity of single compounds and complex mixtures of various origins like body fluids, foods, beverages, plant extracts [21].

TABLE II
ABTS RADICAL CATION DECOLORIZATION OF FIVE SELECTED ETHNOMEDICINAL PLANTS

Herbal Plant	ABTS antioxidant activity mg TE/g	SD
M P L	0.362	± 0.006
PNL	0. 283	± 0.008
CPL	0.052	± 0.001
EPL	0.005	± 0.005
TAL	0.056	± 0.002

The antioxidant activity is expressed as Trolox equivalents per gram of dried leaves. Values expressed as mean ± S.D. in three replicates.

$ABTS^+$ scavenging ability reported as the Trolox equivalent antioxidant capacity (TEAC) is presented in Table II. Results revealed that the $ABTS^+$ scavenging ability of the five herbal plants extract were significantly different ($p<0.05$) in the order of MPL>PNL>CPL> EPL >TAL. This is a clear indication that MPL, with TEAC of 0.362 mg TEAC/g dried leaves had a better $ABTS^+$ scavenging ability among other medicinal plants in the study.

C. Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay measures the reducing ability of antioxidants present in a sample and is based on the ability of analyte to reduce the Fe^{3+} to Fe^{2+} pair. The electron donating anti-oxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions. Many studies show that the reducing power of substances is closely related to antioxidant activity [22]. Total antioxidant power can be detected using FRAP assay. The greater the reducing power the stronger the antioxidant activity.

TABLE III
FRAP ACTIVITY OF THE FIVE SELECTED ETHNOMEDICINAL PLANTS

Herbal Plant Extract	FRAP antioxidant activity mg GAE/g	SD
MPL	27.497 ±	0.806
PNL	28.417 ±	1.020
CPL	4.611 ±	0.174
EPL	0.304 ±	0.021
TAL	8.716 ±	0.194

Ferric reducing activity is expressed as Gallic acid equivalents per gram of dried leaves. Values expressed as mean ± S.D. in three replicates.

In this study the reducing power is reported as gallic acid equivalent which is presented in Table III. Results revealed that MPL, PNL, CPL, EPL and TAL were all able to reduce Fe (III) to Fe (II). Considering in this assay the following decreasing order was established: PNL>MPL>TAL>CPL>EPL. The FRAP activity of the PNL is higher than the other medicinal plant.

IV. Conclusion

The results of the present study revealed that the five medicinal plants do not have equivalent antioxidant powers. The relative high values for *M. pudica* (MPL) and *P. niruri* (PNL) supports the medicinal value of the two plants. The total phenolics, ABTS and FRAP correlate strongly with one another as far as the five plants are concerned.

Acknowledgment

The financial support granted by the research section of Surigao del Sur State University, Cantilan and the assistance given by Noel Dael and Mary Grace Lubangco, Analytical Chemistry section to conduct the antioxidant activity studies are appreciatively recognized.

References

[1] F. Pourmorad, S. J., Hosseinimehr and N. Shahabimajd, "Antioxidant activity, Phenol and flavonoid contents of some selected Iranian medicinal plants", African Journal of Biotechnology, vol. 5 (11), 2006, pp. 1142-1145.

[2] L. A. Pham-Huy, H. He and C. Pham-Huy, "Free Radicals, Antioxidants

in disease and health" International Journal of Biomedical Science, 2008 June, 4(2): 89-96.
[3] G. Parameswari and M. Suriyavathana,"*In-vitro* antioxidant activity of *Chromalaena odorata* (L.) King and Robinson", International Research Journal of Pharmacy vol. 3 (11), 2012, pp. 187-192.
[4] N. C. Cook and S. Samman, "Flavonoids- chemistry, metabolism, cardioprotective effects, and dietary sources" Nutritional Biochemistry, 1996, 7: 66- 76.
[5] JT Kumpulainen and JT Salonen," Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease", The Royal Society of Chemistry, UK, 1999, pp 178- 187.
[6] B. Halliwell, JMC Gutteridge and CE Cross," Free radicals, antioxidants and human disease: where are we now? J Lab Clin Med. 1992; 119:598–620.
[7] O. O. Igbinosa, E. O. Igbinosa and O. A. Aiyegoro, "Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn)", African Journal of Pharmacy and Pharmacology, February, 2009,Vol. 3(2). pp. 058-062.
[8] S. Y., Wang, & H. Jiao, "Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry", Journal of Agricultural and Food Chemistry, 2000, 48, 5672–5676.
[9] S. E. Lee, J. H. Hyun, J. -S., Ha, H. S., Jeong & J. H. Kim, "Screening of medicinal plant extracts for antioxidant activity" Life Sciences, (2003), 73, 167–179.
[10] I. Biskup, I. Golonka, A. Gamian and Z. Sroka, "Antioxidant activity of selected phenols estimated by ABTS and FRAP methods", Postepy Hig Med Dosw (online), 2013; 67: 958-963.
[11] S. Vinotha, I. Thabrew, S. Sri Ranjani , "*In vitro* Antioxidant Activity of Two Selected Herbal", Medicines World Academy of Science, Engineering and Technology International Journal of Medical, Health, Biomedical and Pharmaceutical Engineering, (2014) Vol:8, No:12
[12] K. Yuan, A. Jia, JL Lu, "Isolation and Identification of phenolic constituents from *Mimosa pudica*", Chin J Chin Materia Medica, 2006; 31: 1029-30.
[13] R. Karuna, S. Reddy, R. Baskar, and D. Saralakumari, "Antioxidant potential of aqueous extract of *Phyllanthus amarus* in rats," Indian Journal of Pharmacology, vol. 41, no. 2, pp. 64–67, 2009.
[14] D. N. Tchamo, G. Cartier, M.G. Dijoux-Franca, E.Tsamo, and A.M. Mariotte, Pharm. Biol., 2001;39, 3, 202–205.
[15] A. Elumalai, N. Mathangi, A. Didala, R. Kasarla, Y. Venkatesh, "A Review on *Ceiba pentandra* and its medicinal features", Asian J. Pharm. Tech. 2012; Vol. 2: Issue 3, Pg 83-86.
[16] EWC Chan, YY Lim, YL Chew, "Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia", J. food Chem, 2007; 102: 1214-1222.
[17] S. Sellappan, CC Akoh," Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions", Journal of Agriculture and Food Chemistry, 2002; 50:5338-5342.
[18] M Oyaizu, "Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine", Jpn. J. Nutri, 1986; 44:307-15.
[19] F. Shahidi, "Natural antioxidants: an overview", Chemistry, health effects and applications. Champaign, IL: AOCS 1997. Press. P. 1-.
[20] T. Osawa, "Novel natural antioxidants for utilization in food and biological systems", Biochemistry of Plant Food-Materials in the Tropics. Japan Scientific Societies Press, Tokyo, Japan, 1994, p. 241-251.
[21] V. Katalinic´, M. Milos, D. Modun, I. Music´,& M. Boban, "Antioxidant effectiveness of selected wines in comparison with (+)-catechin". Food Chemistry, (2004), 86, 593–600.
[22] X. Duan, G. Wu, Y. Jiang," Evaluation of antioxidant properties of phenolics from litchi fruit in relation to pericarp browning prevention". Molecules, 2007; 12:759-771.

Open Science Index, Bioengineering and Life Sciences Vol:9, No:9, 2015 publications.waset.org/10002103/pdf