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Solvent Effect on Antioxidant Activity and Total Phenolic Content of *Betula alba* and *Convolvulus arvensis*

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***Abstract*—**The potential of using herbal *Betula alba* (BA) and *Convolvulus arvensis* (CA) as a natural antioxidant for food applications were investigated. Each plant extract was prepared by using pure ethanol, different concentration of ethanol aqueous solutions, including 50% and 75%, 50% methanol aqueous and water. Total phenolic content (TPC) was determined using Folin–Ciocalteau method and antioxidant activity were analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, trolox equivalent antioxidant capacity (TEAC), Oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) respectively. Ethanol extract of CA exhibited the highest TPC and antioxidant activity; however BA showed varies of antioxidant activity value in each assay. The BA and CA exhibit the potential sources of natural antioxidant for food commodities.

***Keywords*—**Solvent effect**,** Antioxidant activity, *Betula Alba, Convolvulus arvensis*, Total Phenolic Content.

I. Introduction

REACTIVE oxygen species (ROS) is an intermediate product occurs from natural biological combustion in the organism respiration process. Excessive of ROS can lead to cumulative damage in proteins, lipids and DNA called as an oxidative stress in the body [1]. This imbalance mechanism promotes aging processes and various diseases in humans including cancer, neurodegenerative diseases, inflammation, and cardiovascular disease. Thus, the consumption of antioxidant foods is believed to be important to create balance between antioxidant and oxidation process for reducing the production of ROS and resulted to healthier biological system [1], [2].

Medicinal plants, fruits and selected herbs are among foods that associated to their natural antioxidant contents. Hence, several methods have been developed to measure the antioxidant activity in plants, including the oxygen radical absorption capacity (ORAC) [3], ferric reducing antioxidant power (FRAP) [4], 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging [5], and trolox equivalent antioxidant capacity (TEAC) [6]. The use of natural plants presents a large source of novel bioactive compounds with different activities. In continuation of our screening programme to search for potential plant, *Betula alba* and *Convolvulus arvensis* are among selected herbal plants to be studied for their potential activities. This study was extended to obtain the best alcohol aqueous solvent for retrieval antioxidant activity in the plant extract.

Betula alba (BA) is also known as *Betula pubescens*, a species of native birch mostly found throughout northern Europe, Asia, Iceland and Greenland [7]. BA was reported to display several biological effects, including anti-viral, anti-parasitic, anti-bacterial, anti-inflammatory activities and anti-cancer to inhibit growth of cancer cells [8]. It has been demonstrated recently that BA is also inhibit effectively against head and neck squamous carcinoma cells [9], [10], leukemia cells and other cells lines [11], [12].

Convolvulus arvensis (CA) is a perennial climber commonly found as a weed throughout in the Nile region, the Libyan desert oasis and Sinai [13]. Reviewing from the previous works, it was mentioned in folk medicine that the CA leaves have a purgative activity used in asthma, jaundice and anti-hemorrhagic [14], [15]. Some phytochemical studies were carried out on CA showed the plant contains alkaloids, phenolic compounds, sterols, resin and sugars. Moreover, CA is also believed to have an anticancer effect [15].

Through many researches done to identify the active compounds of BA and CA, this study is aim to evaluate the phenolic content (TPC) and antioxidant activities of BA and CA. The study is extended by determining the best solvent extract between concentration of 100%, 75%, 50% (v/v) of ethanol aqueous, water and 50% (v/v) methanol aqueous on their phenol contains and antioxidant activities. Antioxidant activities of the plants were measured by improved method of TEAC [6], FRAP assay [4], ORAC [3] and a modified DPPH assay [5]. Furthermore, we are also estimated the phenol contents of these plants using the classical Folin–Ciocalteu reagent [16]. This study proved the potential of BA and CA as a source of edible natural antioxidants that can be used by the food industry as an alternative to synthetic antioxidants.

II. Materials and Methods

A. Materials

Commercial dried BA and CA were kindly supply from Manatial de la Salut, a registered herbal company in Barcelona, Spain. All reagents and solvents used were

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analytical grade and obtained from Panreac (Barcelona, Spain) and Sigma Aldrich (England).

B. Extraction of Plant

Dried BA and CA was cleaned and cut and grounded using standard kitchen food processor (Moulinex). Fine grounded plants (2g) were extracted aqueous solvent (v/v) with either i) 100% ethanol, ii) 75% ethanol, iii) 50% ethanol iv) H_2O v) 50% methanol, in the ratio 1:30 (w/v). All extraction were performed at 4±1°C, light protected for 24 hours and constantly stirred at 1000 rpm. Each extraction was carried out triplicates. Then, the extractions were centrifuge to separate the supernatant and the extracts were stored in -80°C for further analysis.

C. Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to determine the total phenolic content as reported by Santas et al. with some modification [16]. Appropriate dilution of antioxidant extracts were mixed with 80µL Folin-Ciocalteu reagent and 2% (w/v) Sodium Carbonate. The mixture was finally diluted with miliQ water, shaked and incubated in the dark for 1 hour. Absorbance 765nm was measured using microplate reader (Fluostar Omega, BMG Labtech, Germany) against miliQ water. Gallic acid was used as a standard calibration; the results were expressed as mg of Gallic acid equivalents (GAE) per g dried weight sample (mg GAE/g DW).

D.FRAP assay

The FRAP method used is from Benzie and Strain (1996) with little modifications [4]. The FRAP solution was prepared in a proportion of 10:1:1 of acetate buffer (300mM) (pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) (10mM) in HCl (40mM) and FeCl3 (20mM), respectively. FRAP solution was incubated at 37°C for 30 minutes before mixing with the appropriate dilution of samples. The absorbance was measured at 593nm using microplate reader. Results were expressed as mg of Trolox equivalents per g dried weight sample (mg TE/g DW).

E. DPPH assay

The effect of extracts on the scavenging of DPPH radicals was determined according to the method adapted from Madhujith and Shahidi with slight modifications [5]. DPPH reagent (0.1mM) was dissolved with MeOH and mixed with different concentration of the samples. Dilution from the extract was mixed with DPPH–methanol reagent and the absorbance was measured at 517nm for 90min. The results were expressed as mg TE/g DW.

F. TEAC assay

The antioxidant capacities of BA and CA were measured by using a modified TEAC assay [6]. The TEAC assay was based on the reduction of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)$^{\bullet-}$ radicals cation ($ABTS^{\bullet-}$) by the antioxidants present in the samples. Appropriate dilutions were prepared for BA and CA. $ABTS^{\bullet-}$ radical cation (7mM) was dissolved before adding potassium sulphate (2.45mM) and allowing the mixture to stand in the dark up to 16 hours. PBS (10 mM) was incubated at room temperature for 30 min before used. Then, the mixture of the $ABTS^{\bullet-}$ radical cation was adjusted to an absorbance of 0.72 ± 0.2nm, using microplate reader (Fluostar Omega, BMG Labtech, Germany). The TEAC values for the different concentrations of each compound were interpolated from the Trolox standard curve. The results expressed as mg TE/g DW.

G. ORAC assay

The ORAC value was determined according to [3] with some amendments. An appropriate concentration of BA and CA extracts were mixed with 13mM phosphate buffer (incubated at 37°C for 20 minutes) and 80mM fluorescein respectively. 60mM APPH radical was added after the initial value of fluorescence was recorded and the fluorescence was monitored for 150 minutes using a microplate reader (Fluostar Omega, BMG Labtech, Germany). The net area under the fluorescein decay curve (AUC) was determined and ORAC values were calculated by comparing the AUC to that of Trolox as a standard. All measured data were expressed as mg of TE/g DW.

H. Statistical Analysis

Differences between solvent extracts determined by analysis of variance (ANOVA) using the least squares difference method of the General Linear Model in SPSS. Differences were significant at $p < 0.05$.

III. Results and Discussion

TABLE I
EXTRACTION YIELD IN DIFFERENT SOLVENT EXTRACTS

[a]Yield (%)	BA	CA
EtOH	4.79 ± 0.18b	1.66 ± 0.01b
75% EtOH.H_2O	10.67 ± 0.52c	6.79 ± 0.26c
50% EtOH.H_2O	8.69 ± 0.32c	6.22 ± 0.14c
H_2O	7.98 ± 0.11c	4.25 ± 0.17c
50% MeOH.H_2O	8.45 ± 0.41c	5.96 ± 0.29c

[a] Extraction yield (%) is calculated according to the method of Zhang and Liu [17]. Assay was carried out triplicates with less 5% of standard deviation error.

b-c different letter indicate significant difference ($p<0.05$)

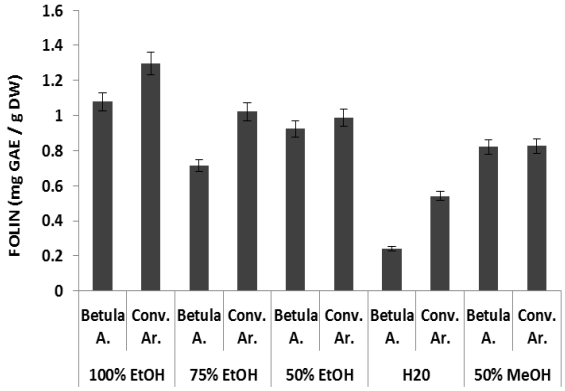

Fig. 1 Total phenolic content of BA and CA in different solvent extracts; expressed in mg Gallic acid equivalent (GAE) per g dried weight sample. Assay was carried out triplicates with less 5% of standard deviation error

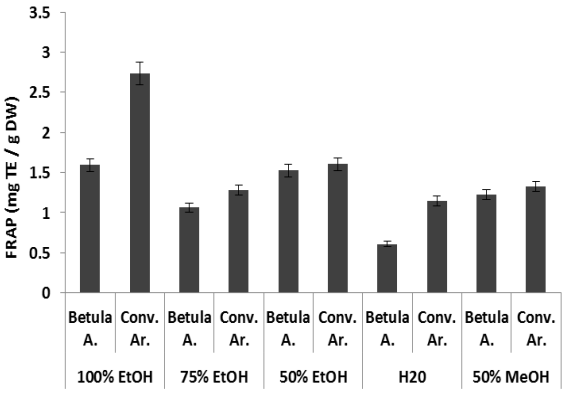

Fig. 2 FRAP assay of BA and CA in different solvent extracts; expressed in mg Trolox (TE) per g dried weight sample. Assay was carried out triplicates with less 5% of standard deviation error

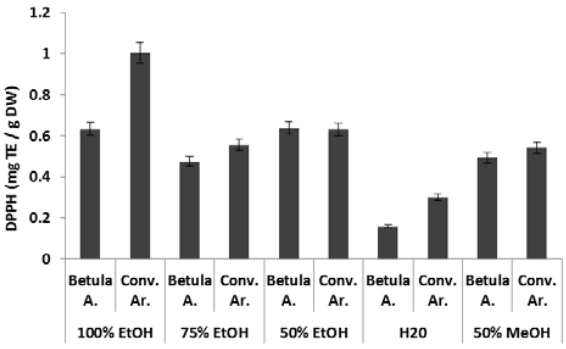

Fig. 3 DPPH assay of BA and CA in different solvent extracts; expressed in mg TE per g dried weight sample. Assay was carried out triplicates with less 5% of standard deviation error

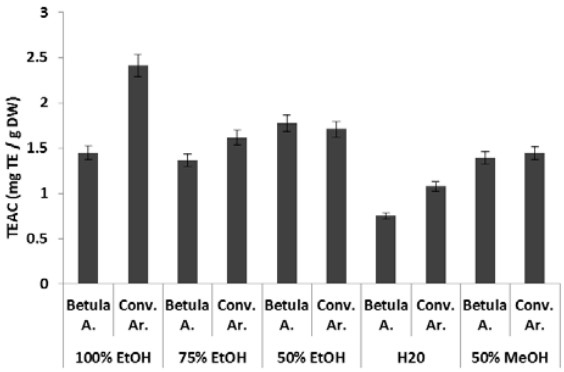

Fig. 4 TEAC assay of BA and CA in different solvents extracts; expressed in mg TE per g dried weight sample. Assay was carried out triplicates with less 5% of standard deviation error

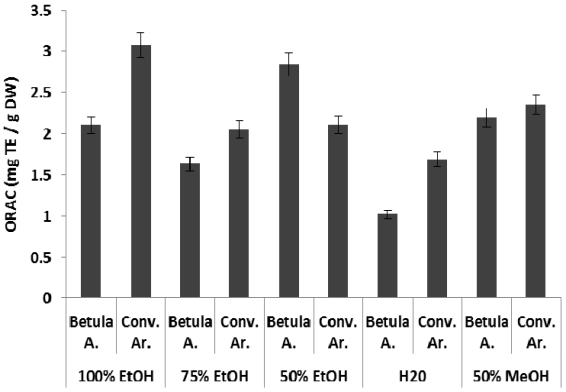

Fig. 5 ORAC assay of BA and CA in different solvent extracts; expressed in mg TE per g dried weight sample. Assay was carried out triplicates with less 5% of standard deviation error

TABLE II
CORRELATION BETWEEN POLYPHENOL CONTENT AND ANTIOXIDANT ASSAY OF BA AND CA

[a]Assay	[b]R^2
FOLIN vs FRAP	0.7488
FOLIN vs DPPH	0.9032
FOLIN vs TEAC	0.8392
FOLIN vs ORAC	0.7086

[a]Correlation between polyphenol content (FOLIN assay) vs antioxidant capacity assay
[c]Good regression values is accepted from $R^2 \geq 0.7$, and the standard deviation for each assay is less than ±0.5.

Several principles have to be considered before making a decision to choose appropriate solvent for plant extraction. Some consideration were according to the purpose of extraction (preparation or analysis), the nature of the assayed components, the physicochemical properties of the matrix, the availability of reagents and equipment, the cost and safety concerns [18]. The different polarity of solvent employed in the extraction had strong association to the yield and antioxidant activity of the natural plant extract [19]. However, the choice of determining the best solvent extraction properties had a lot to consider. The selection must consider

each element of assorted structure and composition of the matrix and complex behavior of each matrix-solvent system which particularly hard to predicted [20]. Thus, in this work, different solvents were assayed for the extraction of BA and CA (water, ethanol, 75% and 50% ethanol aqueous solutions and 50% ethanol aqueous) on the extraction yield, total phenols content and antioxidant activities. Total phenolic content and antioxidant capacity assay were carried out three times in each assay; the values were determined by means of different assay. In all determinations, the percentage of standard deviation was accepted must be lower than 5%.

Table I shows the results obtained of BA and CA extraction yields correspond to their solvent extracts. There are no significantly different on the extraction capacities between aqueous solvents used ($p<0.05$). The highest value of the extract yield obtained in 75% ethanol aqueous of BA with 10.67 ± 0.52. The extraction yield increase in order: ethanol < water <50% methanol <50% ethanol <75% ethanol. In addition, the increased or decreased of extraction yields were depended on the solvent polarity of extraction [21]. The mixtures of alcohols and water have been more efficient in extracting compounds and better yield than the corresponding mono-component solvent system.

The Folin-Cioalteau method had been employed for many years to determine the total phenols in natural products. Although there are some interfering substance in the method such sugars, aromatic amines, sulphur dioxide and ascorbic acid, this method was most popular to determine the total phenol content in the plant sample [22]. Fig. 1 shows the total phenolic content for BA and CA in different solvent extract. The extracts with the highest total phenols content were obtained with 100% ethanol in BA and CA, followed by CA in 75% ethanol and BA in 50% ethanol extracts. The lowest value was obtained by water extract. There is almost the same phenol content of CA and BA in 50% methanol extracts with the value of 0.81 ± 0.1. The significant different was determined by ethanol and water extraction for both plants ($p<0.05$). Many reports claimed the used of binary solvent was the most favourable for extraction of phenolic compounds from plants compared to mono-solvent systems, however, the claimed was contrasted in this study which showed ethanol extract showed the highest phenol content for BA and CA [23], [24].

Extraction of active compound in natural plants is potent to protect biological system against damaging effect of natural oxidation process in organism. Thus, potential of antioxidant activity of the compounds extract can be evaluate by various antioxidant activity assay in this study. In this study, the antioxidant of BA and CA in different solvent extract was evaluate by 4 assays; DPPH, FRAP, TEAC and ORAC. Each antioxidant assay possesses its own unique mechanism to evaluate the antioxidant activity in sample.

FRAP method was used to present rather quick and simple method measuring antioxidant presents in the BA and CA. The FRAP assay is based on the ability of phenolics to reduce yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (FE(II)-TPTZ) by the action of electron-donating antioxidants [4]. The result of blue colour measured spectrophotometrically at 593 nm is taken as linearly related to the total reducing capacity of electron-donating antioxidants. As shown in Fig. 2, ethanol extract showed significant different with all solvent used in this study ($p<0.05$). Absorbance values measured in the extracts varied from 0.61 to 2.66 mg TE / g DW. The aqueous solvent did not exhibit any significant different with water in FRAP method ($p>0.05$). CA extract with pure ethanol showed the highest antioxidant activity in FRAP assay exhibited the significant different from all solvents used. BA extract with water showed the lowest activity however, it was found high antioxidant capacity in both ethanol and ethanol aqueous extraction. In most instances, the ethanol aqueous solvent of BA and CA extracts contained substantial ferric reducing activities compared to the methanol aqueous and water extracts.

BA and CA extracts scavenging ability were measured by DPPH assay. The DPPH• radical is one of the few stable organic nitrogen radicals and the test is simple and rapid which probably explains its widespread use in antioxidant screening [5], [22]. In this method, the purple chromogen radical DPPH• is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine [25]. The loss of DPPH colour after reaction with test compounds was monitored at 517 nm. The discoloration indicates the scavenging potential of the extract, overall, all the extract of BA and CA were able to decolorize DPPH. The activity result was similar to FRAP assay. BA showed lower value compared to CA in water, and methanol aqueous solvents. Nevertheless, CA and BA displayed similar value of antioxidant activity in 50% ethanol extract with 0.63 ± 0.02 mg TE / g DW ($P<0.05$). The DPPH values are measured based on the reducing ability of the plant extract towards DPPH radicals. The extracts obtained with ethanol showed the highest antioxidant activity of CA. Ethanol and ethanol aqueous shows no significant different in BA extract ($p<0.05$)

In TEAC assay, CA extracts displayed higher antioxidant value than BA as similar to FRAP and DPPH assays ($p<0.05$). Nevertheless BA extract in 50% ethanol (1.75 ± 0.05 mg TE / g DW) was the only measurement gives higher antioxidant than CA (1.69 ± 0.13 mg TE / g DW) ($p<0.05$). The assay indicted the plants potency and potential use as a source of antioxidants based on the ability of antioxidants compound to scavenge the long-life radical cation $ABTS^{+}$. The radical anion $ABTS^{\bullet-}$ is generated by $ABTS^{2-}$ oxidation by potassium persulfate. The radical is stable and formed non color diamagnetic compound when reacts by electron transfer with antioxidant [6]. As Fig. 4 shows CA ethanol extract and BA extract in 50% ethanol aqueous had the highest capacity to scavenge ABTS radicals and consequently CA in ethanol extract shows the highest antioxidant activity in FRAP and DPPH as well.

ORAC assay measure the capacity for active compound in plant to scavenge peroxyl radicals generated by spontaneous

decomposition of AAPH radicals [3]. The measurement value was estimated in terms of Trolox equivalents similar to TEAC, DPPH and FRAP. This assay applied to a wide variety of different phytochemicals from edible plants, purified or as an extract or fraction including alkaloids, coumarins, flavonoids, phenylpropanoids, terpenoids, and phenolic acids [22]. Among the plant extracts assayed here, the values were found to be in the range between 3.33 ± 0.13 to 1.69 ± 0.06 mg TE / g DW of CA and 2.86 ± 0.11 to 1.03 ± 0.07 mg TE / g DW of BA. CA showed highest antioxidant activity in ethanol and BA extract in 50% ethanol aqueous. 50% methanol extract in CA showed higher activity compared to aqueous ethanol extracts although is not significant ($p<0.05$). The ability of BA and CA extract to scavenge peroxyl radical was showed in ORAC assay and gives the highest value of all antioxidant assays.

The antioxidant assays (FRAP, DPPH, TEAC and ORAC) and FOLIN values of the extracts correlated well, with $R^2 > 0.7$ (Table II). The FRAP and FOLIN correlation shows the lowest value compare with $R^2 > 0.7488$, meanwhile DPPH reported to have the best correlation with phenolic compound with $R^2 > 0.9032$. Several studies have compared different methods, to evaluate the antioxidant activity of samples, although a general consensus has not yet been established. Some authors find similar values between the methods while others report noticeable differences between them or a dependency on the type of food sample. Nevertheless the good correlations between phenol content and antioxidant assays confirm that phenols are mainly responsible for the antioxidant activity of extracts. Some study has reported a good correlation between the phenol content of plant extracts and antioxidant activity [16]. Differences in antioxidant activity determined by different methods emphasise the importance of using several methods to assess the parameters in order to obtain accurate data and to improve comparison with other literature.

The different measurement values among antioxidant assay were attributed to the different chemistry principle upon the basis of each method. The DPPH, FRAP and ABTS methods are based on a single electron transfer (SET) reaction. In these methods, antioxidants are oxidised by oxidants, such as a metal (Fe III) or a radical (DPPH or ABTS+). As a result, a single electron is transferred from the antioxidant molecule to the oxidant. In contrast, the ORAC assay is based on a hydrogen atom transfer (HAT) reaction. The HAT is transferred after a peroxyl radical ROO has been generated in which this radical extracts a hydrogen atom from the antioxidant compounds. Furthermore, the ORAC assay only measures the activity of chain-breaking antioxidants against peroxyl radicals [22], [25]. Therefore there was relative difference in the measurement of antioxidant assay respectively. The values demonstrated the ability of BA and CA extract either to quench peroxyl radicals or to reduce radicals generated in the assay.

Overall, the influence of the solvent used on extract properties was the same for the total phenols content and antioxidant assays. Extraction of CA in ethanol exhibited the highest total phenolic content and antioxidant activity assay respectively. Ethanol resulted to be effective in the extraction of flavonoids and their glycosides, catecols and tannins from raw plant materials [26]. Unlike methanol or other strong solvent for extraction such as acetone or chloroform, ethanol is recognised as a GRAS (Generally Recognised as Safe) which positively can be used for applications in the food industry [21]. However, BA extracts with ethanol and ethanol aqueous showed no significant different on the antioxidant activity values in each antioxidant assay and total phenolic content. ORAC assay showed good scavenging values of BA extract with 50% ethanol.

Determination of the best solvent extract for BA and CA with measurement of TPC and various antioxidant activity assays was one important factor to increase extraction process efficacy. To the best of our knowledge, this study was the first time reported the best solvent extract of BA and CA for determination of their phenolic compound and antioxidant activity. BA and CA are known in many physiological functions and attracted economic interested to the food industry. These plants were valued for its nutritional, health and sensory attributes however, require more research on their antioxidant activity. Betulinic acid is a pentacyclic triterpene, one of active compound in BA which displayed range of biological effects, including antiviral, antiparasitic, and antibacterial [9], [10], [27]. It is also useful as anti-inflammatory activities, and in particular to inhibit growth of cancer cells [8], [28]. Methyl syringate compound isolated from BA was antifungal, proved to inhibit for aflatoxin; the most dangerous contaminants occurs in food and feed [29]. CA was believed to have antidiarrhoeal activity [14], cytotoxic effect against a number of tumor cells, immunostimulant effect, anti-bacteria and anti-tumor, anti-angiogenic properties [30]. GC-MS and HPLC results showed the presence of flavonoids, p-hydoxybenzoic acid, syringic acid, vanillin, benzoic acid and ferulic acid were responsible for the antioxidant activity [14]. Moreover, the preliminary literature suggested that *Betula alba* and *Convolvulus arvensis* were having their unique compounds for the benefit of neutraceutical, pharmaceutical and medicinal used. Thus, this research was extended to evaluate the antioxidant capacity of these plants as a primary study for alternative source of natural antioxidant for food commodities.

IV. Conclusion

In summary, this study has revealed that a range of values for total antioxidant capacities and phenolic contents exist among the *Betula alba* and *Convolvulus arvensis.* Varies of measurement related to different solvent concentration in the extraction and which antioxidant activity assay had been performed. Overall, CA displayed the highest antioxidant activity in ethanol extract and BA shows varies measurement in antioxidant assays and total phenolic content respectively. The good correction between antioxidant assays and total

phenolic content were determined for BA and CA extracts. Thus, this study was performed useful to evaluate the antioxidant capacity in various assays that supports the initial study for its potential sources of potent natural antioxidant

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