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**“Challenges of Biotechnological Research in  
Food and Health”**

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November 15<sup>th</sup>, 2014**

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FACULTY OF TECHNOLOGY AND FOOD INDUSTRY  
SLAMET RIYADI UNIVERSITY  
SURAKARTA**

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# POTENCY OF BELUNTAS (*Pluchea indica* Less) LEAVES EXTRACT AS ANTIOXIDANT AND ANTI WARMED OVER FLAVOR (WOF) OF DUCK MEAT

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## Abstract

Beluntas (*Pluchea indica* Less) is a herb plant used as a traditional medicine or eaten in fresh form. There are phytochemical compounds such as essential oils, flavonoids, phenolics, tannins, saponins, phenols hydroquinone, and cardiac glycosides compounds of beluntas leaves that cause it having potential as antioxidant. Difference of solvent polarity can cause concentration and composition of phytochemical compounds in extract differed, therefore this study was conducted to determine the potential for beluntas leaves extracts (water, methanol, ethanol, ethyl acetate, and hexane) and fractions (water, ethyl acetate, and n-butanol) as an antioxidant and antiwarmed over flavor (WOF) in duck meat during storage. The results showed that the methanol extract of the beluntas leaves (EMB) of the most potential as a source of antioxidants because the concentration and composition of phytochemical compounds, total phenols and total flavonoids than the water, ethanol, ethyl acetate, and hexane extracts. Furthermore EMB was fractionated by difference of solvent polarity (ethyl acetate, water, n-butanol). Test showed that EMB antioxidant capacity and its fractions had the difference in the ability of antioxidant compounds in the EMB and each fraction in different test systems. EMB had the potency to scavenge superoxide radicals, reduce iron ions, and inhibit bleaching of linoleic acid- $\beta$ -carotene system. Ethyl acetate fraction (FEA) had the potency to scavenge superoxide radicals, reduce iron ions, chelate of iron ions and haemoglobin (Hb), thus FEA was effective as antiwarmed over flavor (WOF) in duck meat, which protected linoleic acid, decreased of TBARS and hexanal.

Keywords: beluntas (*Pluchea indica* Less), antioxidant, antiwarmed over flavor, duck meat

## Abbreviations:

EMB :Beluntas methanol extract  
FEA :Ethyl acetate fraction  
DPPH : 2,2-diphenyl-1-picrylhydrazyl radical  
AT : Alpha tocopherol  
WOF : Warmed over flavor  
Hb : Haemoglobin  
AC :Control absorbance  
AS : Sample absorbance  
UV-Vis : Ultra violet-visible  
GAE : Gallic acid equivalent  
CE : Catechin equivalent  
IC : Inhibition concentration  
TBARS : Thiobarbituric acid reactive substances  
BHT : Butyl hydroxyl toluene  
DM : Fresh duck meat  
DP : Cooking duck meat  
DPSP : Warmed stored cooking duck meat  
MDA :Malondialdehyde

## 1. Introductions

Beluntas (*Pluchea indica* Less), herb plant is a species in the family *Asteraceae*, used as a traditional medicine or eathen in fresh form (Ardiansyah et al. 2003). Beluntas leaves contain phytochemical compounds such as lignin, ter-

pene, phenylpropanoid, benzoid, alkanes, sterol, 2-(prop-1-unyl)-5,5,6-dihydroxy hexa-1,3-diunyl)-thiophene, (-)-catechin, phenol hydroquinone, saponin, tannin, and alkaloid, flavonol (quercetin, kaempherol, myricetin, luteoline, apigenine) (Andarwulan et al., 2010). Generally, phytochemical compounds, especially phenolic in plants have free structure or glycoside and ester bonds (Dehkharghanian et al., 2010). There are hydroxyl groups at phenolic structure caused these compounds having polar properties (Lai et al., 2009). Water, ethanol, and methanol are polar solvent that can extract glycoside structure of phenolic compounds. Ethyl acetate has potentially to extract alkaloid, aglycon, and glycoside compounds (Houghton and Raman, 1998), phenolic compounds with low until high molecular weight (Mariod et al., 2010), and then hexanes can extract lignin, wax, lipid, aglycon, sterol, and terpenoid (Houghton and Raman, 1998). Previous research had found that aqueous extract of beluntas leaves can scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, superoxide and hydroxyl radicals, iron reducing power, and iron ion che-

lating. Ethanolic extract of beluntas leaves can scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and 2,2'-azino-bis- (3-ethyl benzo thiazoline- 6-sulphonic acid (ABTS) cation radical, iron ion reducing power, and inhibit bleaching linoleic acid- $\beta$ -carotene system (Andarwulan et al., 2010). Methanol is effective to extract phenolic compounds with low molecular weight having medium polarity and results the highest yield and antioxidant activity (Chan et al., 2007). Nevertheless until now ethyl acetate and hexanes haven't known antioxidant activity. Fractionation of beluntas leaves extract with several solvent different polarity, such as ethyl acetate, water, and n-butanol is done to result yield with different antioxidant compound composition (Lai et al., 2009), so that can resulted extract or fraction the most potentially as antioxidant source. Each solvent can solve phytochemical compound with different composition. n-butanol can extract polar compounds, i.e. glycoside, aglycon, dan gula (Liu dkk., 2011). WOF (*Warmed over flavor*) is flavor degradation at food product because of food product warmed again after they are stored in refrigerator at 4-5°C for 48 hours (Jayathilakan et al., 2007). WOF can be happened by lipid oxidation at meat and heme protein denaturation that can accelerate lipid oxidation (Masqood and Benjakul, 2011). Duck meat can be oxidized to result WOF because it contains high lipid at adipose tissue. Until now antioxidant capacity of beluntas leaves extract and its fractions haven't known to inhibit WOF so that it is studied. Beluntas leaves extract (EMB) and its fractions can reduce synthetic antioxidant using at food product because of carcinogenic (Valentao et al., 2010). The research was done to study the potency of beluntas leaves extracts (water, methanol, ethanol, ethyl acetate, and hexanes) and its fractions (water, ethyl acetate, and n-butanol) as antioxidant and anti warmed over flavor at duct meat for storage.

## 2. Methods

### 2.1. Materials

Beluntas leaves are harvested at Dramaga area, Bogor and Sukolilo area, Surabaya. Green tea is purchased at *Tea Factory* in Singapura (Lim Lam Thye PTE, LTD). Dried Rosemary is purchased at *Cold storage supermarket* in *Holland Evanue*, Singapura. Chicken blood Darah ayam is cultivated from Laladon traditional market, Bogor. Chemical is used Analytical grade from Sigma, Merck, JT Beaker, and Riedel-de Haen, except ethanol (PT Brataco) and aquadest from Microbiology laboratory, SEAFast-PAU-IPB.

### 2.2. Preparation of beluntas leaves extracts and its fractions

Extraction of beluntas leaves was based on Dorman dan Hiltunen (2004) modification method. 1-6 segment beluntas leaves sorted, washed, and dried at ambient temperature for a week were milled with 40 mesh. And then beluntas leaves flour was analysed moisture content and extracted with different solvent polarity i.e. water, methanol, ethanol, ethyl acetate, and hexanes with 1:15 b/v ratio for 3 hours at boiling point. Extract was evaporated by rotary evaporator at 40°C. And then extract was analysed yield, phytochemical compounds, and antioxidant activity. Extract having the highest antioxidant activity was fractionated by different solvent polarity (ethyl acetate, n-butanol, and water). And then fraction was evaporated at 40°C and analysed yield, phytochemical compounds, and antioxidant activity.

### 2.3. Moisture Content

Dried beluntas leaves were measured moisture content based on AOAC (1990) method.

### 2.4. Yield

Yield was determined by Ljubuncic et al. (2005) method that measured by compared with beluntas leaves extract or its fractions weight and dried beluntas leaves.

### 2.5. Assay of Phytochemical Compounds

Phytochemical was determined based on Harborne (1996) method.

### 2.6. Assay of Total Phenol

Total phenol was measured by Manian et al. (2008) method using Folin ciocalteus phenol reagent. Data was stated as mg gallic acid equivalent (GAE) per 100 gram sample dry base. Solution absorbance was measured by spectrophotometer UV-Vis at  $\lambda$  760 nm.

### 2.7. Assay of Total Flavonoid

Total flavonoid was determined by Manian et al. (2008) method using  $\text{NaNO}_2$ - $\text{AlCl}_3$ - $\text{NaOH}$  reagent, data was stated by milligram catechin equivalent (CE) per 100 gram sampel dry base. Solution absorbance was measured by spectrophotometer UV-Vis at  $\lambda$  510 nm.

### 2.8. Assay of DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity was measured based on Manian et al. (2008) method modification. Decreasing of purple color solution was determined by spectrophotometer UV-Vis at  $\lambda$  517 nm after reaction occurred 30 min. Prosentase of inhibition (%) was calculated based on =  $[(AC-AS)/AC] \times 100\%$ , where AC = control absorbance, AS = sample absorbance.



### 2.9. Assay of Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity was determined by Manian et al. (2008) method. Superoxide radical was resulted by NBT-NADH-PMS reaction. Solution absorbance was measured by spectrophotometer UV-Vis at  $\lambda$  560 nm. Prosentase of inhibition (%) was calculated by =  $[(AC-AS)/AC] \times 100\%$ , where AC = control absorbance, AS = sample absorbance.

### 2.10. Assay of Hydroxyl Radical Scavenging Activity

Hydroxyl Radical Scavenging Activity was measured by Manian et al. (2008) method. Hydroxyl radical was resulted by Fe-EDTA-DMSO-ascorbic acid system reaction. Yellow color intensity of solution was measured by spectrophotometer UV-Vis at 412 nm after 15 min was reacted. Prosentase of inhibition (%) was calculated by =  $[(AC-AS) / AC] \times 100\%$ , where AC = control absorbance, AS = sample absorbance.

### 2.11. Assay of Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity was determined by Manian et al. (2008) method. Hydrogen peroxide scavenging capacity was shown by absorbance reduction at 230 nm. Inhibition prosentase (%) was calculated by =  $[(AC-AS) / AC] \times 100\%$ , where: AC = control absorbance and AS = sample absorbance.

### 2.12. Assay of Iron Ion Reducing Power

Iron Ion Reducing Power was measured by Manian et al. (2008) method. Potency of antioxidant compounds reduced iron (III) to iron (II) ion was shown by spectrophotometer Uv-Vis at  $\lambda$  700 nm. Increasing of blue color solution showed increasing of reducing power of antioxidant compounds.

### 2.13. Assay of Haemoglobin (Hb) Chelating Activity

Haemoglobin chelating activity was determined by Bate-Smith (1977) method. Absorbance of red color solution was measured by spectrophotometer UV-Vis at  $\lambda$  578 nm. Increasing of absorbance was correlated increasing of Hb chelating activity. Hb chelating activity (%) was calculated by =  $[(AC-AS)/AC] \times 100\%$ , where AC = control absorbance, AS=sample absorbance.

### 2.14. Assay of Iron (II) Ion Chelating Activity

Iron (II) ion chelating activity was measured by Manian et al. (2008) method. Decreasing of magenta color solution because of  $Fe^{2+}$ -Ferrozine complex was detected by spectrophotometer Uv-Vis at  $\lambda$  562 nm. Chelating activity (%) =  $[(AC-$

$AS)/AC] \times 100\%$ , where AC = control absorbance, AS = sample absorbance.

### 2.15. Assay of Linoleic acid- $\beta$ -Carotene Bleaching Capacity

Linoleic acid- $\beta$ -carotene bleaching capacity was measured by Manian et al. (2008) method. Decreasing of orange color solution because of  $\beta$ -carotene oxidation was detected by spectrophotometer UV-Vis at  $\lambda$  470 nm. Bleaching capacity (%) =  $[(AC-AS)/AC] \times 100\%$ , where AC=control absorbance, AS = sample absorbance.

### 2.16. Analysis of TBARS (Thiobarbituric Acid Reactive Substances)

Oxidation reaction of duck meat after antioxidant compounds were added was detected by Tarladgis et al. (1960). Absorbance of colored solution was measured by spectrophotometer UV-Vis at 532 nm. Data was stated by mg MDA/kg lipid based on TEP (1,1,3,3-tetra ethoxy propanes) as standard compound.

### 2.17. Analysis of Fatty Acid Composition

Fatty acid composition was analysed by 2 steps, i.e. lipid extraction based on Folch et al. (1957) method and methylation based on IUPAC (1987) method. And then methyl ester from fatty acid was analysed by GC-MS (Agilent GC-MS 5975C, Palo Alto, CA, USA). Methyl ester of fatty acid was detected based on time retention of margaric acid (C170) standard. Data was stated by mg fatty acid/g lipid. And then they were determined as fatty acid concentration (%). Fatty acid composition was identified based on mass spectrum database (NIST Library, mass spectra searching program, versi 5, Ringoes, NJ, USA).

### 2.18. Analysis of Hexanal

Hexanal of duck meat was determined by SDE (*Simultaneous steam distillation-solvent extraction*) Likens-Nickerson based on Benca and Mitchela (1954) method. Extract was added  $Na_2SO_4$  anhydrous to reduce water. And the sample was analysed by GC-MS (Agilent GC-MS 5975C, Palo Alto, CA, USA) equipped with mass selective detector (MSD). Mass spectra interpretation was based on mass spectra standard of mass spectra library collected NIST (Nationale Institute Standar and Technology). Data was stated by mg hexanal /kg lipid.

## 3. Results and Discussions

### 3.1. Moisture Content, Yield, Total Phenol, Total Flavonoid, and Phytochemical Compounds

Extraction of 1-6 segment beluntas leaves having  $14.19 \pm 0.17\%$  dry base moisture content with

different solvent polarity was shown at Table 1. Difference of solvent polarity determined yield, phytochemical composition, total phenol, total flavonoid. Yield of water extract was higher (41.40%) than it of the other polar solvent (methanol 38.77%, ethanol 31.66%), semipolar solvent (ethyl acetate 33.58%), and non polar (hexanes 30.00%). Yield informed that bioactive compounds content of beluntas leaves were polar properties, it is supported by Dehkharghanian et al. (2010).

Nevertheless total fenol of methanol extract was higher concentration than the other extract, it is supported by Chan et al. (2007) research that methanol is effective to extract plant phenolic and inhibit polyphenol oxidase that can be influenced to antioxidant activity. Different solvent using for extraction can remove phenolic compounds profile in extract.

Fractionation of methanol extract with different polarity solvent was informed that the order extracted compounds were very polar properties > semipolar > polar, respectively. It was shown that yield of water > ethyl acetate > n-butanol, respectively. The methanol extract contains aglycon, glycoside, amino acid, and sugar (Houghton and Raman, 1998). However phenolic compounds contained in methanol extract had semipolar properties because ethyl acetate fraction had the highest total phenol

(126.97±1.61 mg GAE/100g sample db). Ethyl acetate was effective to extract phenolic compounds with low until high molecular weight and medium polarity (Mariod et al., 2010) in aglycon and glycoside structure (Houghton and Raman, 1998). Qualitative analysis of phytochemical of methanolic extracts and its fractions was shown at Table 2. Methanol extract had the most quantitative phytochemical, it was correlated to total phenol and total flavonoid contents. Difference of color intensity showed difference of phytochemical compounds that was caused by difference of solvent polarity.

### 3.2. DPPH Free Radical Scavenging Activity

Data at Figure 1 showed that beluntas methanol extract (EMB) had the highest DPPH free radical scavenging activity compared with the other extracts (water, ethanol, ethyl acetate, and hexanes), it was correlated with total phenol and total flavonoid.

Kubola and Siriamornpun (2008) also informed that there are correlated among total phenol, total flavonoid, and DPPH free radical scavenging activity. Figure 2. showed that EMB and ethyl acetate fraction (FEA) had DPPH free radical scavenging activity compared with control antioxidant (green tea methanol extract, rosemary methanol extract and BHT).

Table 1. Yield, Total Phenol, and Total Flavonoid of Various Beluntas Extracts and Beluntas Methanol Extract's Fractions

Component	Extract Type					Beluntas Methanol Extract's Fractions		
	Water	Methanol	Ethanol	Ethyl Acetate	Hexanes	Water	Ethyl Acetate	n-Butanol
Yield (%)	41.40	38.77	31.66	33.58	30.00	44.54	30.48	14.87
Total Phenol (mg GAE/100g db)	12170±1096	145445±6677	16958±897	1829±166	481±35	42.58±1.08	126.97±1.61	55.63±0.41
Total Flavonoid (mg CE/100g db)	5305±459	157471±6148	18555±1792	2637±187	1150±94	41.54±5.45	57.11±2.11	38.49±0.56

Table 2. Phytochemical Compounds of Various Beluntas Extract and Beluntas Methanol Extract's Fractions

Phytochemical	Extract Type					Beluntas Methanol Extract's Fractions		
	Water	Methanol	Ethanol	Ethyl Acetate	Hexanes	Water	Ethyl Acetate	n-Butanol
Sterol	-	+++	++++	+++	++	-	++	+
Flavonoid	+++	+++++	++++	++	+	++	++++	++
Tannin	+	++++	++	-	-	++	+++	+
Phenolic	+++	+++++	++++	++	++	++	++	+

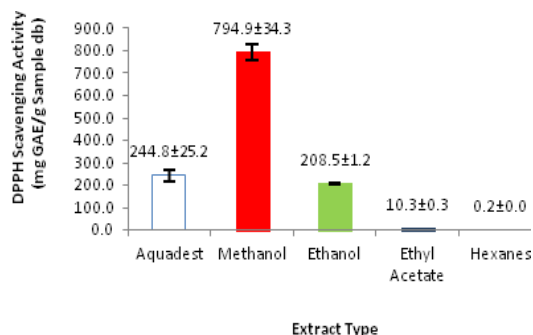
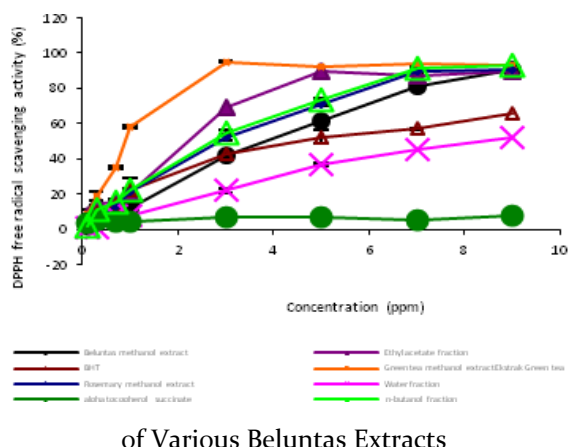


Figure 1. DPPH Free Radical Scavenging Activity



of Various Beluntas Extracts

Figure 2. DPPH Free Radical Scavenging Activity among Beluntas Methanol Extract, Its Fractions and Control Antioxidant

EMB contained semipolar, polar, and very polar phytochemical compounds, whereas FEA of EMB was only arranged semipolar phytochemical compounds (Houghton and Raman, 1998). They were potentially to scavenge DPPH free radical because of hydrogen atom donating capacity to DPPH\* to be DPPH-H. Huang et al. (2010) explained that semipolar antioxidant compounds can scavenge higher DPPH free radical than it of polar and very polar antioxidant compounds.

### 3.3. Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity of EMB ( $IC_{50}$ =2.5 mg/L) > alpha tocopherol ( $IC_{50}$ =3,3 mg/L) > FEA ( $IC_{50}$ =6.2 mg/L) > BHT ( $IC_{50}$ =9.1 mg/L). EMB dan FEA were more potentially as superoxide radical scavenging than control antioxidant (BHT and alpha tocopherol) (Figure 3). Phenolic compounds of EMB and FEA could donate hydrogen atom to superoxide radical to form stable oxygen triplet. Effectivity of phenolic compounds as antioxidant because these compounds can form

radical stabilized by resonance (Tapas et al., 2008).

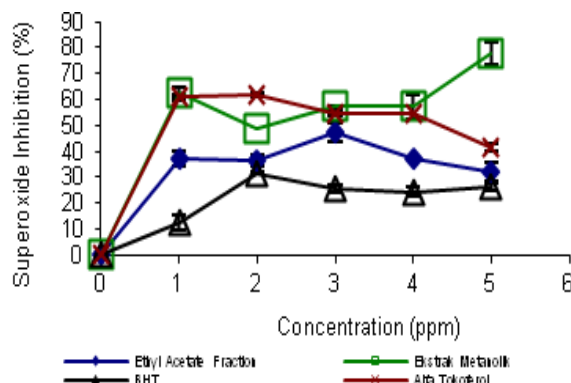
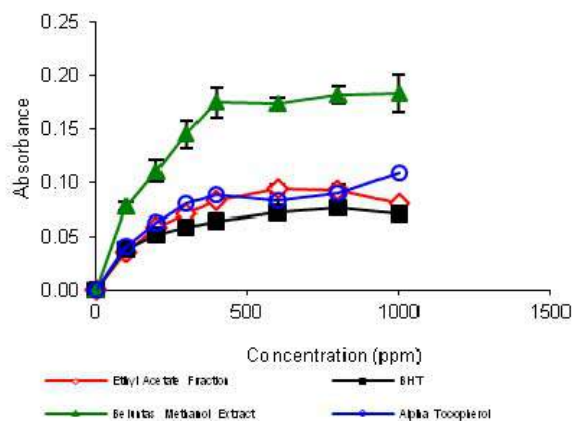


Figure 3. Superoxide Radical Scavenging Activity of EMB, Its Fractions and Control Antioxidant



### 3.4. Hydrogen Peroxide Scavenging Activity

EMB ( $IC_{50}$  = 1575.3 mg/L) and FEA ( $IC_{50}$  = 3404.1 mg/L) were less potentially as hydrogen peroxide scavenging than control antioxidant (BHT  $IC_{50}$ = 608.3 mg/L and alpha tocopherol (AT)  $IC_{50}$ =666.2 mg/L) (Figure 4).

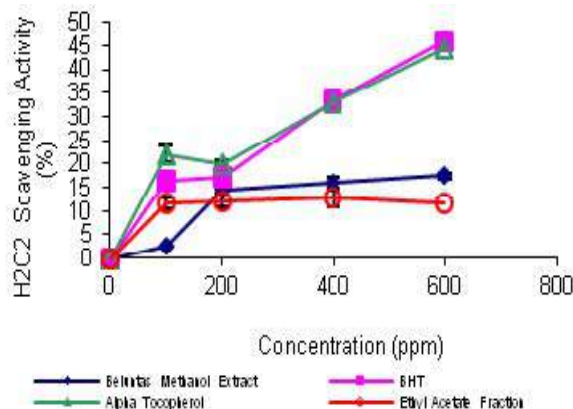


Figure 4. Hydrogen Peroxide Scavenging Activity of EMB, Its Fractions, and control antioxidant.

### 3.5. Iron Ion Reducing Power

Reducing power of EMB was higher significant than it of FEA, BHT, and alpha tocopherol, whereas reducing capacity of FEA, BHT, and alpha tocopherol was the same (Figure 5). Capacity of antioxidant reduced  $Fe^{3+}$  ion to  $Fe^{2+}$  ion was caused by capacity of these compounds to donate electron and result green bluewish solution (Liu et al., 2011). Manian et al. (2008) informed that the existence of reductons in extract and fraction can cleavage free radical chain with hydrogen atom donating so that they can reduce  $Fe^{3+}$  ion.

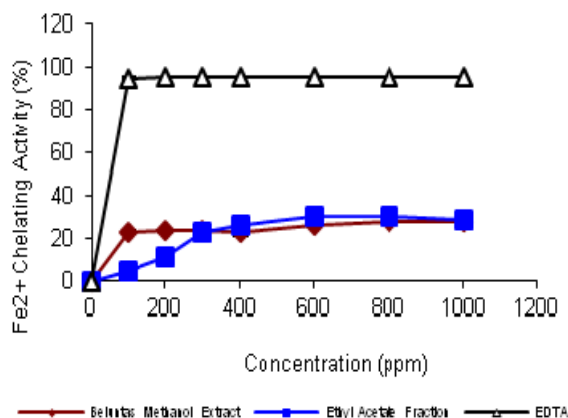


Figure 5. Iron Ion Reducing Power among EMB and Its Fractions, and Control antioxidant

### 3.6. Haemoglobin (Hb) and $Fe^{2+}$ Ion Chelating Activity

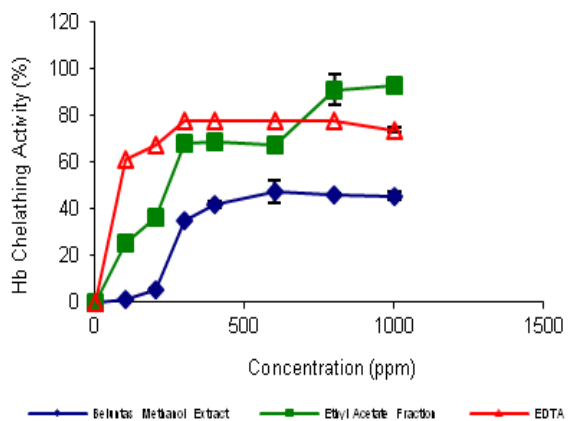


Figure 6.  $Fe^{2+}$  Ion Chelating Activity among EMB, FEA, and Control Antioxidant

Hb and  $Fe^{2+}$  chelating activity of FEA were higher than it of EMB and lower than it of ethylene diamine tetra acetic acid (EDTA) (Figure 6 dan 7). Hb and  $Fe^{2+}$  Ion chelating activity were

predicted to involve interaction between tannin and protein globin. Iron ion chelating activity was determined by functional groups position. Bidentat ligan was stronger metal bonding than it of monodentat. Glycoside formed phenolic compounds are difficult to bond with metal (Wong et al., 2006).

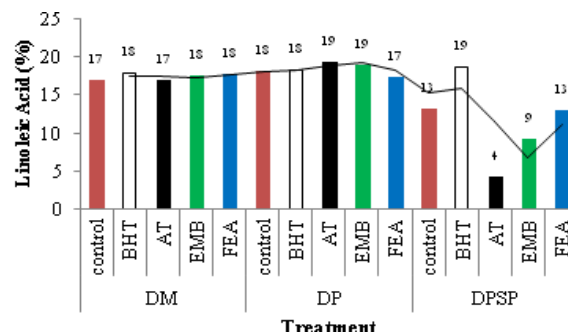


Figure 7. Hb Chelating Activity among EMB, FEA, and Control Antioxidant

### 3.7. Linoleic Acid - $\beta$ - Carotene Bleaching Inhibition

EMB ( $IC_{50}=32.0$  mg/L) had Linoleic acid- $\beta$ -carotene bleaching inhibition capacity similar to AT ( $IC_{50}=30,6$  mg/L), but its activity was lower than BHT ( $IC_{50}= 2,5$  mg/L) and higher than FEA ( $IC_{50}= 46.0$  mg/L) (Figure 8).

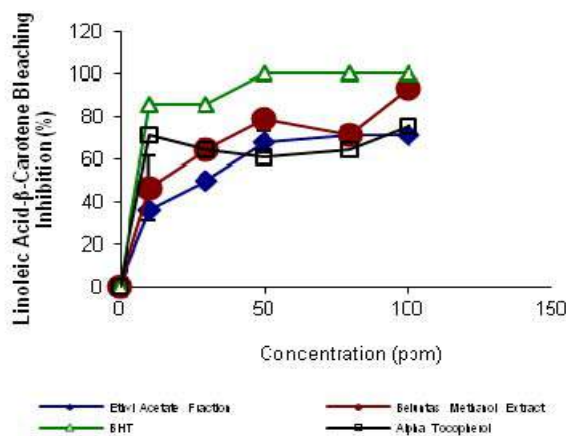


Figure 8. Linoleic Acid- $\beta$ -Carotene Bleaching Inhibition among EMB, FEA, and Control Antioxidant

EMB had potentially to inhibit prooxidation reaction because of involvement of phenolic and non phenolic compounds that had hydrophilic and hydrophobic groups, its prediction is supported by Manian et al. (2008). Zhu et al. (2011) also informed that linoleic acid inhibition capacity can involve many antioxidant mechanism reaction, such as free

radical scavenging activity, metal chelating activity, peroxide decomposition, and chain reaction cleavage that involves all components in extract.

### 3.8. Linoleic Concentration of Duck Meat

Linoleic acid changes of duck meat in various treatment were shown at Figure 9. Linoleic acid oxidation can result hexanal (Varlet et al., 2007), that can be used as warmed over flavor (WOF) of meat and its products (Pignoli et al., 2009). Linoleic acid changes of fresh duck meat (DM) and cooking duck meat (DP) were still low, but the effect was seen significant of warmed stored cooking duck meat (DPSP). 250 ppm FEA addition could inhibit linoleic acid oxidation compared with control (EMB 250 ppm and AT 300 ppm), but its activity is lower than it of BHT 200 ppm. Lee *et al.* (2006) said that BHT is synthetic antioxidant that can prevent lipid oxidation with oxidation chain cleavage from free radical.

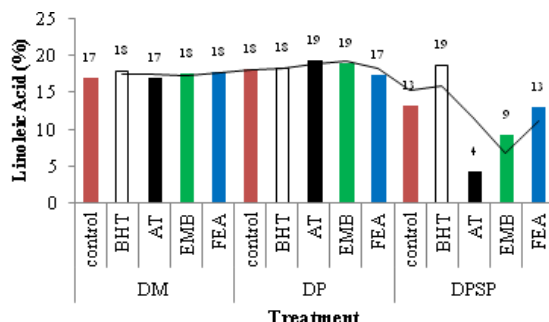


Figure 9. Linoleic acid of various treatments of duck meat (DM = fresh duck meat, DP = cooking duck meat, and DPSP = warmed stored cooking duck meat).

Huang *et al.* (2010) informed that linoleic acid oxidation is supported by heme iron ion and free radical. FEA was more potential to chelate free and bind iron ions at haemoglobin and reduce iron ion (Ganhao *et al.*, 2011).

### 3.9. TBARS (Thiobarbituric Acid Reactive Substances) of Duck Meat

Lipid oxidation at duck meat for treatment was shown by malondialdehyde (MDA) formation (Pignoli *et al.*, 2009), that could be stated with TBARS (*thiobarbituric acid reactive substances*) (Figure 10). The antioxidant addition can reduce significant TBARS compared with control antioxidant.

Heating and storage can increase significant TBARS, whereas warmed of DPSP sample can

reduce TBARS, it is predicted that decomposed MDA or interaction between MDA and protein. The antioxidant addition of DM could reduce significant TBARS because of interaction between MDA and phenolic groups (Varlet *et al.*, 2007). DPSP showed oxidation reaction of linoleic acid (Figure 9). The addition of 250 ppm FEA could prevent MDA formation because the semipolar phytochemical compounds could dissolve in duck meat that was matrix complex arranged polar and non polar compounds (Huff-Lonergan and Lonergan, 2005).

### 3.10. Hexanal at Duck Meat

Hexanal at duck meat was formed by linoleic acid oxidation reaction (Figure 11). Data showed that hexanal had been formed at all samples. Concentration of this samples was still low at DM, the data was supported by linoleic acid concentration (Figure 9) and TBARS formation (Figure 10).

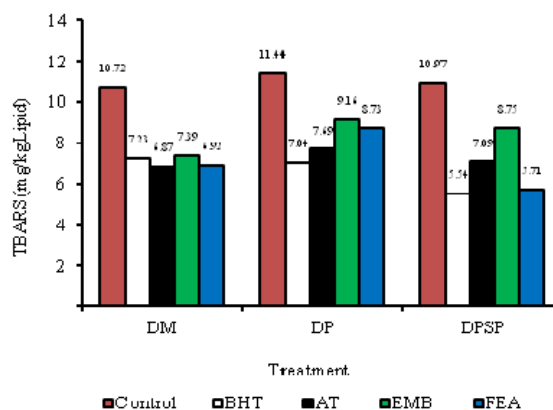


Figure 10. TBARS of duck Meat at various treatment (DM = fresh duck meat, DP = cooking duck meat, DPSP = warmed stored cooking duck meat)

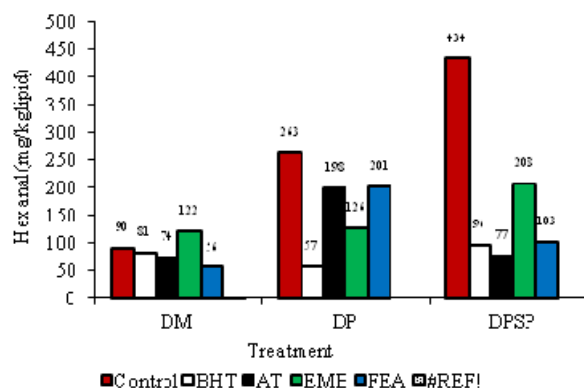


Figure 11. Hexanal of duck meat at various treatment (DM = fresh duck meat, DP = cooking duck meat, DPSP = warmed stored cooking duck meat)

Fatty acid oxidation increases because of heating, storage, and warmed over. FEA addition could inhibit more linoleic acid oxidation than EMB. Difference of phytochemical content of FEA and EMB caused their difference of antioxidant activity. Phytochemical compounds of EMB were semipolar until polar properties, whereas they of FEA were semipolar properties.

#### 4. Conclusions

EMB was potentially as antioxidant source because its concentration and composition of phytochemical compounds, total phenol, and total flavonoid were higher than their of water, ethanol, ethyl acetate, and hexanes extracts. The antioxidant capacity of EMB and its fractions were different at various of assay system. EMB was potentially to scavenge superoxide radical, reduce of iron ion, inhibit of linoleic acid- $\beta$ -carotene bleaching. FEA was potentially to scavenge superoxide radical, reduce of iron ion, chelate of metal ion and Hb so that FEA was potentially as antiwarmed over flavor (WOF) of duck meat, such as maintainance of linoleic acid, reducing of TBRAS, and decreasing of hexanal.

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#### REFERENCES

- Andarwulan N *et al.* Short communication flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chemistry* 2010; 121 : 1231-1235.
- [AOAC] Association of Official Analytical Chemist. *Official methods of analysis of association of official analytical chemists*. Edition ke-15. USA ; Kenneth Helrich; 1990.
- Ardiansyah, Nuraida L, Andarwulan N. Antimicrobial activity of beluntas leaves (*Pluchea indica Less*) and its stability at various of salt concentration and pH levels. *Jurnal of Technology and Food Industry* 2003; 14(2) : 90-97.
- Bate-Smith EC. Astringent tannins of Acer species. *Phytochemistry* 1977; 16 : 1421.
- Benca HMF, Mitchela JH. Estimation of carbonyls in fats and oils. *Journal of American Oil and Chemists Society* 1954; 31 : 88-96.
- Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of *Etligeria* species (*Zingiberaceae*) in Peninsular Malaysia. *Food Chemistry* 2007; 104 : 1586-1593.
- Dehkharghanian M, Adenier H, Vijayalakshmi MA. Analytical methods study of flavonoids in aqueous spinach extract using positive electrospray ionisation tandem quadrupole mass spectrometry. *Food Chemistry* 2010; 121 : 863-870.
- Dorman HJD, Hiltunen R. Fe(III) reductive and free radical-scavenging properties of summer savory (*Satureja hortensis L.*) extract and sub-fractions. *Food Chemistry* 2004; 88 : 193-199.
- Folch J, Lees M, Sloan-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 1957; 226 : 497-507.
- Ganhão R, Estévez M, Morcuende D. Analytical methods suitability of the TBA method for assessing lipid oxidation in a meat system with added phenolic-rich materials. *Food Chemistry* 2011; 126 : 772-778.
- Harbone JB. *Phytochemical method*. Padmawinata K, Soediro I, translator. Bandung : Institute of Bandung Technology, Indonesia; 1996.
- Houghton PJ, Raman A. *Laboratory Handbook for the Fractionation of Natural Extracts*. New York : Chapman & Hall; 1996.
- Huang B *et al.* Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf. *Meat Science* 2010; 87(1) : 46-53.
- Huff-Lonergan E, Lonergan SM. Mechanisms of water-holding capacity of meat : The role of postmortem biochemical and structural changes. *Meat Science* 2005; 71 : 194-204.
- Jayathilakan K, Sharma GK, Radhakrishna K, Bawa AS. 2007. Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. *Food Chemistry* 2007; 105 : 908-916.
- Kubola J, Siriamornpun S. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia L.*) leaf, stem, and fruit fraction extracts in vitro. *Food Chemistry* 2008; 110 : 881-890.
- Lai P, Li KY, Lu S, Chen HH. Analytical methods phytochemicals and antioxidant properties of solvent extracts from Japonica rice bran. *Food Chemistry* 2009; 117 : 538-544.
- Lee S *et al.* Effect of antioxidants and cooking on stability of n-3 fatty acids in fortified meat products. *Journal Food Science* 2006; 71(3) : C233-C238.
- Liu J *et al.* The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays L.*) and related flavone

- glycosides. *Food Chemistry* 2011; 126 : 261–269.
- Ljubuncic P *et al.* Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel, *Journal of Ethnopharmacology* 2005; 99 : 43–47.
- Manian R, Anusuya N, Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chemistry* 2008; 107 : 1000–1007.
- Mariod AA, Ibrahim RM, Ismail M, Ismail N. Antioxidant activities of phenolic rich fractions (PRFs) obtained from black mahlab (*Monechma ciliatum*) and white mahlab (*Prunus mahaleb*) seedcakes. *Food Chemistry* 2010; 118 : 120–127.
- Masqood S, Benjakul S. Retardation of haemoglobin-mediated lipid oxidation of Asian sea bass muscle by tannic acid during iced storage. *Food Chemistry* 2011; 124 : 1056–1062.
- Pignoli G, Bou R, Rodriguez-Estrada MT, Decker EA. Suitability of saturated aldehydes as lipid oxidation markers in washed turkey meat. *Meat Science* 2009; 83 : 412–416.
- Tapas A, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. *Tropical Journal of Pharmaceutical Research* 2008; 7(3) : 1089–1099.
- Tarladgis BG, Watts BM, Younathan MT. A distillation method for the quantitative determination of malondialdehyde in rancid foods. *Journal of American Oil and Chemists Society* 1960; 37 : 44–48.
- Valentão P *et al.* *Codium tomentosum* and *Plocamium cartilagineum*: chemistry and antioxidant potential. *Food Chemistry* 2010; 119 : 1359–1368.
- Varlet V, Prost C, Serot T. Analytical, nutritional and clinical methods volatile aldehydes in smoked fish : Analysis methods, occurrence and mechanisms of formation. *Food Chemistry* 2007; 105 : 1536–1556.
- Wong SP, Leong LP, Koh JHW. Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* 2006; 99 : 775–783.