A study on a potential bioactive compound in green seaweed *Chaetomorpha antennina* Kützing (1847) extract as antioxidant from the Gosong Telaga Coast, Aceh Singkil

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Abstract. Seaweed has considered as herbal medicine and food sources utilized by the coastal community to overcome their health problem. Aceh Singkil is one of the regency in Aceh Province that this surrounding area always be affected by oceanography phenomena such as tidal, wind, season, climate change and others. Aceh Singkil coastal area have high marine biodiversity especially seaweed. Seaweed are commonly categorized into three main classes, including Rhodophyta, Chlorophyta, and Phaeophyta. *Chaetomorpha antennina* is one of the green seaweed that widely distributed on the Aceh coastal area. The objective of this study was to identify the potential active compound of *C. antennina* toward antioxidant activity. The sample of *C. antennina* was collected from around Gosong Telaga coastal zone. The research phase including rendemen, extraction, phytochemical screening, and antioxidant activity. The data depicted the content of rendemen in ethanol extract as much as 1.98%, ethyl acetate extract was 0.66%, and n-hexane extract was 1.08%. It is indicated that the ethanol extract has high rendemen content than other extracts. Subsequently, green seaweed *C. antennina* was detected phenol, flavonoid, and steroid compounds. The cupric reducing antioxidant capacity method were obtained the ethanol extract was 44.7 umol trolox/g, ethyl acetate was 13.84 umol trolox/g, and n-hexane extract was 29.02 mmol trolox/g. Moreover, antioxidant activity with FRAP method yielded the ethanol extract value 576.50 umol trolox/g, the value of ethyl acetate as much as 500.50 umol trolox/g, and n-hexane extract value 200.50 mmol trolox/g. Based on two antioxidant activity that used in this study depicted the ethanolic crude extract have the strongest antioxidant activity than other extracts. This finding gives fruitful report for developing the marine natural product from green seaweed.
1. Introduction

Indonesia is an archipelagic state with megabiodiversity in the world after Brazil [1] that has various potential marine biota as a source of marine natural products for herbal drug and cosmeceutical. Seaweed is a marine plant that grows in the marine environment with unique thallus that attached in the substrate, such as dead coral, sandy, rocky, or other bases in coastal zones. Seaweed is a group of plant that widely distributed in intertidal and subtidal zones that possesses many benefits for human’s health. Mostly seaweed utilized by the coastal community because it has high economic fisheries products. According to seaweed’s classification was categorized into three divisions, such as Rhodophyta, Chlorophyta, and Phaeophyta. Nowadays, enormous novel metabolites with potential biopharmac product have been found from the marine biota. One of the richest sources of structurally diverse marine natural products turned out from seaweed [2-4]. One of the renewable resources with various active compound with nutritional value and display a therapeutic properties from seaweed [5, 6] and also great prospective for application in the biopharmac, foodstuff, nutraceutical, and cosmetical factory. These species are rich in bioactive component, water-soluble, fat-soluble, dietary fibers, proteins, polyunsaturated fatty acids, and mineral [7-11]. A number of the novel component has been assayed from seaweeds, and many of them have been reported to possesses great secondary metabolites [12, 13].

The green seaweed is the most diverse group of algae, it belongs to the class Chlorophyceae with more than 7000 species growing in a variety of coastal zone. The green seaweed is a ‘paraphyletic’ group because it excludes the Plantae [14]. According to Subathraa and Poonguzhali [15] that green seaweed has been repeatedly utilized as a natural material to extract bioactive components because of their widespread distribution and large biomass in the coastal zone. The green algae contain two forms of chlorophyll, which they use to capture light energy to fuel the manufacture of sugars [16]. The globalization era, the antioxidant activity in seaweed extracts has received attention for their important role to prevent the human diseases. The antioxidant substances in seaweeds including alkaloids, flavonoids, phenols, tannins, phorotannin, terpenoids, pigments, glycosides and steroids was thought to act as a defense mechanism, protecting them against reactive oxygen species (ROS) resulting from environment effect [17,18]. The reactive oxygen species (ROS) in the metabolism of a living organism are produced and eliminated by the defense system of the enzymatic and non-enzymatic. Nevertheless, the defense system can fail and accumulated by ROS as well as other free radicals can cause irreversible damage to proteins, amino acid, lipids, and DNA if under stressful conditions. Therefore The reactive oxygen species (ROS) has been related with several diseases that damage human health [19-21]. The presence of antioxidants in seaweed protected the species structural components from environmental oxidative damage [22]. In addition to the damage to cellular components, ROS can also promote the degradation of oils and fats present in foods, leading to the appearance of odors and rancid flavor, which contributes to decrease the quality and nutritional security in view of the formation of potentially toxic secondary metabolites [23, 24]. In the modern era, many antioxidant are added to foodstuff or drugs to pressure the oxidative deterioration [25] and widely utilized the chronic diseases prevention [26]. Nevertheless, mostly the synthetic antioxidant that circulated in the market has contained toxin and exert carcinogenic effects such as butylhydroxyanisole (BHA), butylhydroxytoluene, and tert butylhydroquinone [27]. Thus, we offer new alternative natural antioxidants for consumers from green seaweeds from coastal area, particularly in the West South Aceh coast. Several previous studies on potential antioxidants of green seaweed from West South Aceh have already done, such as Halimeda macroloba [28], Chaetomorpha crassa [29], Halimeda opuntia [30], Halimeda sp [31]. Nevertheless, the recent study on the antioxidant activity of Chaetomorpha antennina of the Gosong Telaga coast has no yet reported. The coast of Gosong Telaga is located in Aceh Singkil regency that in front of the Hindian ocean with various seaweeds that distributed widespread alongside the coastal area. Based on the background above, the aim of this research was to evaluate the antioxidant activity of green seaweed C. antennina extract from the coast of Gosong Telaga.
2. Material and Method

2.1. Sample collection and Preparation
Samples of green seaweed *C. antennina* (Kützing) were collected in April 2017 at the Gosong Telaga beach, Aceh Singkil Regency, Aceh province (Figure 1). Collected samples were determined in the Laboratory of Mathematic and Natural Science, University of Teuku Umar. The samples collection were the firstly rinsed with flowing water and remove all impurities, sand particles, and attached biota manually.

![Figure 1. Collection samples site](image)

2.2. Chemical and Reagents
All chemical used in this research such as 4,6-tri(2-pyridyl)-s-triazine (TPTZ), Dragendorff’s reagent, mercuric chloride, ascorbic acid, potassium iodide, iodine were purchased from Sigma–Aldrich, ethanol pa (Merck), methanol pa (Merck), ethyl acetate pa (Merck), n-hexane pa (Merck) hydrochloric acid (HCl), sulfuric acid (H$_2$SO$_4$), chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH), CuCl$_2$.6H$_2$O (Merck), FeCl$_3$.6H$_2$O (Merck) and potassium peroxodisulfate. The instrument used in this study such as vacuum rotary evaporator, glassware (pyrex) and UV-VIS spectrophotometer.

2.3. Extraction of green algae *C. antennina*
The dried samples of *C. antennina* were minced and milled by using a blender until it became powder. Subsequently, the dried simplicia were weighed 60 gram and put the simplicia in the glass. The maceration was conducted with 1:3 by using three solvents such as n-hexane, ethyl acetate, and methanol and it was soaked for 3x24 hours. The solution were filtered used Whatman paper 42 and then these filtrates were concentrated in a vacuum rotary evaporator.

2.4. Phytochemical assay
Phytochemical assay in this research with crude methanolic extract of dried sample *C. antennina* were tested phytochemical properties including phenol, alkaloids, steroid and triterpenes, flavonoids, saponins, and tannins. The qualitative result is expressed as (+) for detected and (-) not detected [31].

2.5. Ferric reducing antioxidant power Method
In FRAP assay, the reagent of fresh FRAP was prepared by mixing 300 mM acetate buffer as much as100 mL, 10 mM from the solution of TPTZ (10 mL), and kept warm at 37 °C used in the experiment.
As much as $300\text{ mM}$ acetate buffer with pH $3.6$ was prepared by dissolving sodium acetate trihydrate ($3.1\text{ g}$) in distilled water ($500\text{ mL}$) then glacial acetic acid ($16\text{ mL}$) was added and made up to the mark of $1\text{ L}$ with distilled water. Trolox was used as the standard and a calibration curve [32], [33]. Standard solution ($150\text{ µL}$) and sample extract ($150\text{ µL}$) in different eppendorf for $30\text{ min}$ in the dark condition. Reading of the colored solution (ferrous tripyridyltriazine complex) of standard and sample was taken in the extract was reported as mg trolox equivalent (TE)/g extract.

2.6. Modified CUPRAC Method
As much as $0.5\text{ mL}$ of $2.10^{-3}\text{ M}$ Cu(II) chloride, $0.5\text{ ml}$ ethanol, $1\text{ ml}$ of $7.5\ 10^{-3}\text{ M}$ neocuproine solution (Nc), $1\text{ ml}$ of $1\text{ M}$ NH$_4$Ac buffer solution (pH 7) was added in $0.5\text{ ml}$ sample and $0.8\text{ ml}$ ethanol to eppendorf to make the final volume. As much as the solutions of $4\text{ Ml}$. Nc and NH$_4$Ac in absolute ethanol. The eppendorf was stoppered, and after half hour, the absorbance at $450\text{ nm} (A_{450})$ was recorded. The cupric ion reducing activity (CUPRAC) method is based on the reduction of a cupric neocuproine complex [Cu(II)-Nc] by antioxidants to the cuprous form [Cu(I)-Nc]. The standard calibration curve of trolox was constructed as absorbance vs. concentration, and the molar absorbptivity of the CUPRAC method for each antioxidant was found from the slope of the calibration line concerned [34]. The bis (2,9-di,ethy-1,10-phenanthroline: neocuproin) Cu(II) chelate cation as the chromogenic oxidant CUPRAC was used in method of total antioxidant capacity (TAC) assay, which is reduced in the presence of antioxidants to the cuprous neocuproine chelate [Cu(I)-Nc] showing maximum light absorption at $450\text{ nm}$. Color in the CUPRAC method is based on the reaction [35]. The cupric ion reducing activity assay result expressed as the Trolox equivalent antioxidant capacity.

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n\text{Cu(Nc)}_2^{2+} + n\text{-e reductant} \leftrightarrow n\text{Cu(Nc)}_2^{+} + n\text{-e oxidized Product} + n\text{H}^+
\]

3. Result and Discussion

3.1. The green seaweed C. antennina characteristic
The green seaweed C. antennina always attached vigorously in wooden as a substrate because of a strong surge from the ocean at the coast of Gosong Telaga Aceh Singkil were in front of the Indian ocean. According to the observation that C. antennina have characteristic with unbranched filament with green color. These species were unique seaweed where is easily identifiable by its characteristic erect, brush-like tuft, composed of the straight and proximal pole of a holdfast cell of a filament (Figure 2).

![Figure 2. The C. antennina morphology from the coast of Gosong Telaga](image-url)
The crude extract rendement was calculated based on the ratio of the final weight (weight of extract produced after evaporation) with the initial weight (weight of biomass used). The crude extract rendement of *C. antennina* was presented in Table 1.

| Crude extracts          | Rendement (%) |
|-------------------------|---------------|
| Ethanol                 | 1.98          |
| Ethyl acetate extract   | 1.08          |
| n-hexane extract        | 0.66          |

Based on the result showed, ethanolic crude extract has the highest rendement value of 1.98% and the ethyl acetate crude extract value of 1.08%. However, the n-hexane crude extract has the lowest rendement value of 0.66%. This is indicated that the polar compound was more dominant that other extracts. Harborne [36] explained that the ethanol is a polar solvent that able to extract active component in extracellular and intracellular liquid.

### 3.3. Phytochemical study

Phytochemical compounds are secondary metabolite groups in living organisms that have a certain function for humans. To know the phytochemical compound, in this research have been detected five types of phytochemical compounds that are estimated to found in green seaweed *C. antennina* (Table 2).

| Phytochemical test          | Ethanol | Crude extract | n-hexane | Positive result According to the Reference |
|-----------------------------|---------|---------------|----------|-------------------------------------------|
| Alkaloids                   | -       | -             | -        |                                           |
| Phenolic                    | +       | +             | +        | The color change occurs from the control tube |
| saponin                     | -       | -             | -        |                                           |
| Triterpenoid/Steroid        | -       | -             | -        |                                           |
| Tannin                      | -       | -             | -        |                                           |

Description: (+) : detected, (-) : not detected

The phytochemical screening of crude extract of *C. antennina* revealed the presence of secondary metabolites, namely flavonoid, as shown in Table 2. The bioactive compound detected in our sample was fruitful preliminary study to continue to the quantitative analysis. For example, fenolic derived from plants are known to have an antioxidant activity.

Polyphenols are a significant group of compounds acting as primary antioxidant. However, the determination of phenolic compounds in the plant extract were justifiable. Polyphenolic compounds have an aromatic benzene ring with a substituted hydroxyl group, including their functional derivatives. These are able to absorb free radical and can chelate metal ions that could catalyze the reactive oxygen species (ROS) formation, which promotes peroxidation of lipid. Among polyphenol, flavonoids have play important rolec to fight against harmful diseases in human body. The flavonoids can be acted as great antioxidant agents depends on their molecular structures, the position of the hydroxyl group, and other features in its structure of chemical [37].

### 3.4. The result of antioxidant with CUPRAC method
The CUPRAC method is based on Cu(II)-Cu(I) reduction by antioxidants in the presence of neocuproine. Determination of antioxidant activity with the CUPRAC method was undertaken with CUPRAC assay used comparative compound, namely routine. Routine compound is made with several concentration ((10 μg/mL, 15 μg/mL, 20 μg/mL, 25 μg/mL dan 30 μg/mL) then added into CuCl₂·2H₂O 0.01 M solution, ethanolic neocuproine 0.0075 and acetate ammonium buffer pH 7 1M. Antioxidant activity was stated as the capacity of CUPRAC reduction was obtained from the result of sample absorbance decreasing to control absorbance [38].

### Table 3. The CUPRAC result

| Crude extracts       | The capacity of antioxidant (mmol Trolox/g extract) |
|----------------------|---------------------------------------------------|
| Ethanolic extract    | 62.25±0.07                                        |
| Ethyl acetate extract| 17.93±0.36                                        |
| n-hexane extract     | 32.38±0.063                                       |

Based on Table 3, the antioxidant assay with the CUPRAC method showed antioxidant activities positively with ethanolic crude extract with a value of 62.25, ethyl acetate crude extract value of 17.93. Meanwhile, n-hexane extract had antioxidant capacity value of 32.38 mmol trolox/g extract. It is indicated that ethanol extract possessed phenolic compounds than other crude extracts. According to Apak et al. [39], the CUPRAC method is much more used to antioxidant capacity for the phenolic compound. Therefore, this ethanolic crude extract was predicted to possess a lot of phenolic compound content.

### 3.5. Ferric ion reducing power (FRAP)

In the second method in this study, we also use the FRAP method which depends upon the ferric tripyridyltriazine (Fe(III)-TPTZ) complex reduction to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reduction at low pH.

### Table 4. The FRAP result

| Crude extract       | The capacity of antioxidant (mmol Trolox/g extract) |
|---------------------|---------------------------------------------------|
| Ethanolic extract   | 576.50± 7.7                                       |
| Ethyl acetate extract| 500.50±0.70                                      |
| n-hexane extract    | 200.50±0.70                                       |

The result of FRAP assay are reported in Table 4 depicted the FRAP values were higher in methanolic crude extract than the ethyl acetate and n-hexane crude extract which in the FRAP value of ethanolic extract was 576.50± 7.7 whereas the ethyl acetate extract of FRAP value of 500.50±0.70 and n-hexane extract value of 200.50±0.70. These results yielded that methanol extraction was more efficient in extracting antioxidants in seaweed compared with ethyl acetate and n-hexane. Hodzic et al. [40] reported, FRAP assay was aimed to determine antioxidant activity quickly and simple. However, some disadvantage this assay of the FRAP does not react fast with some antioxidants like glutathione [41]. The higher FRAP values indicate higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the agent of reducing.

Moure et al. [42] stated the natural extracts quality and antioxidant activities does not only depend on storage time, geographic origin, harvesting date, but also environment and technological factors as well. Besides that, the solvents are used in our experimental research is one of the determinat factors in antioxidant compounds extraction in plant materials due to the different antioxidant potential of
component with different polarities. The light penetration and temperature also affect antioxidant activity during storage.

4. Conclusion
This study concluded that the ethanolic crude extract of green seaweed C. antennina possesses marine drugs properties because the phenolic component that contained in green seaweed C. antennina compared to other crude extract and as it also shows antioxidant activities in ethanolic crude extract greater than others. Thus, it could be utilized as a source of antioxidants.

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References
[1] Hernani H 2011 Pengembangan Biofarmaka Sebagai Obat Herbal Untuk Kesehatan Buletin Teknologi Pascapanen Pertanian 7(1) 20-29
[2] Wijesekara I, Pangestuti, R and Kim S K 2011 Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae Carbohydr Polym 84 14-21
[3] Guven K C, Percot A and Sezik E 2010 Alkaloids in marine algae Mar Drugs 8 269-284
[4] El-Gamal A A 2010 Biological importance of marine algae Saudi Pharm J 18 1-25
[5] S M M El Ahwany 2016 Bioactivity and phytochemical constituents of marine red seaweeds (Jania rubens, Corallina mediterranea and Pterocladia cappilacea ) J Taibah Univ Sci 10 (4) 471-484
[6] I S Fernando K A, Sanjewaa K W, Samarakorn W W, Lee H S, Kim E A, Kim, Gunasekara D, Abeytunga C, Nanayakkara, E de Silva 2017 FTIR Characterization and antioxidant activity of water soluble crude polysaccharides of Sri Lankan marine Algae Algae 32 (1) 75-86
[7] Matanjun P, Mohamed S, Mustapha N.M, Muhammad K. 2009 Nutrient content of tropical edible seaweeds. Eucheuma cottonii, Caulerpa lentifera and Sargassum polycystum J Appl Phycol 21 75-80
[8] Pattara R F, Paiva L, Neto A I, Lima E, Baptista J 2011 Nutritional value of selected macroalgae J App Phycol 23 205-208
[9] Pires-Cavalcante KMS Alencar DB, Sousa MB, Sampaio AH Saker-Sampaio S 2011 Seasonal changes of α-tocopherol in green marine algae (Caulerpa genus) J. food Sci 76 (5) : 775-781
[10] Sousa M B, Pires K M S, Alencar D B, Sampaio A H., Saker-Sampaio S 2008 α-β-caroteno e α-tocopherol em algas marinhas in natura Cienc Technol Aliment 28(4). 953-958
[11] U K Prospective Diabetes Study Group 1998 Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33) Lancet 352 837-53
[12] Bailey C J and Day C 1989 Traditional plant medicine as treatments for diabetes Diabetes Care 12 553-64
[13] Harsha K, Joshi D D, Preethi Panthari, Manish Kant Pant, Amit C Kharkwal 2012 Algae as future drugs Asian J. Pharm and Clin Res 5 (14); 23-30
[14] Subathraa K and T V Poonguzhali 2013 Effect of different extracts of Chaetomorpha antennina and their phytochemical screening Int J Curr Scie, 35-39
[15] S Aseer Manilal, Sugathan Sujith, Joseph Selvin, Mamkootahil Velayuthan, Nataraja Panikar, Shiney George 2012 Anticoagulant potential of polysaccharide isolated from the Indian red algae, Asparagopsis taxiformis (Delile) Trevisan Int J Mar Scie 9-15
[16] Yuan Y V and Walsh N A 2006 Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds Food chem. Toxicol 44 1144-1150
[17] Senguttuvan J, Paulsany S and Karthika K 2014 Phytochemical analysis and evaluations of leaf and root parts of the medicinal herb, Hypocharis radicata for in vitro antioxidant activities Asian Pac J Trop Med 9 372-379

[18] Boisvert C, Beauleu L, Bonnet C, Pelletier E 2015 Assessment of the antioxidant and antibacterial activities of three species of edible seaweeds J Food Biochem 39 (4): 377-387

[19] O’ Sullivan AM. O’ Callaghan YC, O’Grady MN, Queguineur B Hannifu D Troy DJ 2011 In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland Food Chem 126 (3): 1064-1070

[20] de Alencar D B, de Carvalho F CT, Rebouças R H, Dos Santos D R, Dos Santos Pires-Cavalcante K M de Lima R L, Baracho B M, Bezerra R M, Viana F A, Silva Dos Fernandes Vieira R H, Sampaio A H, de Sousa O V, Sampaio S S 2016 Bioactive Extracts of Red Seaweeds Pterocladiella capillacea and Osmundaria obtusiloba (Floridophyceae: Rhodophyta) With Antioxidant and Bacterial Agglutination Potential Asian Pac J Trop Med 9(4):372-379

[21] O’ Sullivan A M, O’ Callaghan YC, O’Grady MN, Queguineur B Hannifu D Troy DJ 2011 In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland Food Chem 126 (3): 1064-1070

[22] Ngo D H, Vo T S, Ngo D N. Wijesekara I, Kim S K 2012 Biological activities and potential health benefits of bioactive peptides derived from marine organisms Int J Biol Macromol 51(4) : 378-383

[23] Tierney M S, Smyth T J, Hayes M, Soler-Vila A, Croft A K, Brunton N 2013 Influence of pressurized liquid extraction and solid-liquid extraction methods on the phenolic content and antioxidant activities of Irish macroalgae Int J Biol Macromol 51(4): 378-383

[24] O’ Sullivan AM. O’ Callaghan YC, O’Grady MN, Queguineur B. Hannifu D Troy DJ et al 2011 In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland Food Chem 126 (3): 1064-1070

[25] Wojcik M, Burzynska-Pedziwiatr I, Wozniak L A 2010 A review of natural and synthetic antioxidant important for health and longevity Curr Med Chem 17 (28) 2362-3288

[26] Cox S, Abu-Ghannam N, Gupta S 2010 An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweed Int Food Res J 17 205-220

[27] M Gazali, Nurjanah, NP Zamani 2019 The screening of bioactive compound of the green algae Halimeda macroloba (Decaisne, 1841) as an antioxidant agent from Banyak Island Aceh Singkil IOP Conf Ser: Earth Environ Sci 348 012043

[28] M Gazali, N P Zamani and Nurjanah 2019 The potency of green algae Chaetomorpha crassa Agardh as antioxidant agent from the coastal of Lhok Bubon, West Aceh IOP Conf Ser: Earth Environ Sci 278 012029

[29] M Gazali, Nurjanah, Neviaty Putri Zamani 2019 Skreeing Alga Hijau Halimeda opuntia (Linnaeus) sebagai Antioksidan dari Pesisir Aceh Barat JIPI 24 (3) 267-272

[30] M Gazali 2018 Aktivitas Inhibitor Tirosinase Rumput Laut Halimeda sp dari Pesisir Aceh Barat Jurnal Perikanan Tropis 5(2): 149-159

[31] Iqbal E, Salim K A, Lim B L L 2015 Phytochemical screening, total phenolic and antioxidant activities of bark and leaf extract of Goniathalamus vetulusinus (Airy Shaw) from Brunei Darussalam. J King Saud University – Sci 27 224–232

[32] Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne D H 2006 Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts J Food Comp Analysis 19 669–675

[33] Gan R Y, Xu XR, Song F L, Kuang L, Li H B 2010 Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases J Med Plants Res 4 2438–2444

[34] Baskan K S, Tutem E, Ozer N 2013 Spectrophotometric and chromatographic assessment of contributions of carotenoids and chlorophylls to the total antioxidant capacities of plant foods J Agric Food Chem 61 11371-81
[35] Karaman Ş, Tütem E, Başkan K S, Apak R 2010 Comparison of total antioxidant capacity and phenolic composition of some apple juices with combined HPLC–CUPRAC assay Food Chem 120 1201-1209

[36] Harborne JB 1984 Phytochemical methods Second Edition Chapman and Hall

[37] Rajanandh M G, Kavitha J 2010 Quantitative estimation of bsitosterol, total phenolic and flavonoid compounds in the leaves of Moringa oleifera Int J Pharm Tech Res 2 1409–1414

[38] Apak R, Guclu K, Ozyurek M, dan Esin C_elik S 2008 Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay Springer-Verlag 160 413–419

[39] Apak R, Guclu K, Ozyurek M, dan Esin C_elik S 2008 Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay Springer-Verlag 160 413–419

[40] Hodzic Z, Pasalic H, Memisevic A, Scrabovic M, Saletovic M. and Poljakovic M 2009 The influence of total phenols content on antioxidant capacity in the whole grain extracts Eur J Sci Res 28 471–477

[41] Guo C, Yang J, Wei J, Li Y, Xu J and Jiang Y 2003 Antioxidant activities of peel, pulp, and seed fractions of common fruits as determined by FRAP assay Nut Res 23 (12): 1719–1726

[42] Moure A, Franco D, Sineiro J, Domínguez H, Núñez, M J and Lema J M 2001 Antioxidant activity of extracts from Gevuina avellana and Rosa rubiginosa defatted seeds Food Res Int 34 (2):103-109