Electrospraying Micronization of Phytochemical Compounds Extract from *Eucheuma cottonii*

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Abstract— **A side from being a raw material for agar, Eucheuma cottonii algae have many other benefits because they contain various phytochemical compounds. The phytochemical compounds in Eucheuma cottonii have many benefits in the industrial and pharmaceutical fields. A method to get phytochemical compounds is through the extraction process. In the traditional method, the extraction is using organic solvents that dangerous to the environment. Therefore, this study used an environmentally friendly hydrothermal extraction method. Extraction was carried out at 160oC and a pressure of 7 MPa. The extraction results were then micronized using electrospraying. The electrospraying process was carried out with precursor solution concentration of 4 and 6% w/v, the applied voltage of 12, 14 and 16 kV, and the distance between tip and collector of 6.8 and 10 cm. The particles produced was characterized by Scanning Electron Microscope (SEM), Thermal Gravimetry Analysis (TGA), and antioxidant efficiency (AE) analysis. The morphological form of particles were spheres with a diameter below 3 µm. The largest AE value was 0.1818 obtained at operating conditions of 6% precursor solution, 10 cm tip distance, 16 kV applied voltage. The Operating conditions did not affect the TGA results.**

Keywords—**Eucheuma Cottonii, Hydrothermal, Electrospraying**

I. INTRODUCTION

ONE type of seaweed cultivated by the community is Eucheuma cottonii (Kappaphycus alvarezii). This type is widely cultivated because its production technology is relatively cheap and easy. Besides being an industrial raw material, this type of seaweed can also be processed into food that can be consumed directly. Eucheuma cottonii contains high amounts of dietary fibres, minerals, vitamins, antioxidants, polyphenols, phytochemicals, proteins, polyunsaturated and fatty acids that may have medicinal uses [1].

Carrageenans not only used for human food applications but also in the pet-food industry. Carrageenans are highly valued as stabilizers, viscosifiers and gelling agents in the food and pharmaceutical industries. Kappa- carrageenan, which can form strong gels in the presence of potassium ions is highly valued in dairy applications, meat products and in pet-foods [2][3].

Extraction is one of the main steps for the recovery and isolation of bioactive phytochemicals from plant materials, before do the analysis. This process is influenced by the nature of chemical, the extraction method employed, sample particle size, as well as the presence of interfering substances. Traditionally, abundant volatile organic solvents, including methanol, ethanol, acetone, chloroform, and ethyl acetate. Organic solvent may result in some environmental problems and higher cost [4]. therefore, this study uses subcritical water as a solvent. Hydrothermal extraction contacted eucheuma cottonii with a liquid stream of hot water flowing to rinse the target compound and may decompose several constituents both in the substrate or in the product depending on conditions. the condition is called hydrothermal. That condition happens when liquid water is at a temperature higher than the normal boiling point and at a pressure higher than the saturation pressure. Hydrothermal extraction only requires water as a solvent. therefore, this method is environmentally friendly compared to other techniques that use organic solvents [5].

The usage of water as a solvent has tremendous benefits as a green extraction solvent because water is not only cheap and environmentally friendly. It is also non- flammable, nontoxic, available in nature and pollution prevention [6]. In addition, water also has the highest specific heat capacity value that facilitates control of exothermic reactions and has a hydrogen bond network that can affect substrate reactivity [7]. water diffusivity will increase when the water dielectric constant decreases due to high temperature and pressure. under these conditions, water can dissolve more non-polar molecules [8].

There are many ways of making particles from solution. The method used in this study is electrospraying. Electrospraying in the pharmaceutical field is acknowledged as an emerging tool for preparing mediated drug delivery systems (nanoemulsions, polymeric nanoparticles, liposomes, nanofibers and so forth) with proven efficiency in various treatments [9][10]. The component of standard electrospraying: Syringe pump, high voltage power supply and collector. Electrospraying uses a strong electric field to break down liquids that contain interesting materials into a continuous flow of finely dispersed particles [11] The size of distribution and the surface morphology of the particles produced by electrospraying can be controlled by adjusting the electrospraying operating conditions and formulation parameters [10]. a liquid jet that is charged, at a certain point, will break into droplets. These primary droplets usually experience a phenomenon called Rayleigh disintegration or Coulomb fission. During their flight to the collector, solvent evaporation makes the primary droplets shrink which leads to an increase in charge concentration so that the primary droplets will eventually break into smaller droplets [12].

II. METHOD

A. Materials

Eucheuma cottonii was obtained from local market in sumenep Madura Island. *Polyvinyl pirrolidone* (PVP), *1,1-diphenyl-2- picrylhydrazyl* (DPPH) were purchased from Sigma Aldrich.

B. Experiment

In this research, sub-critical water extraction (SWE) was conducted in a semi-batch process. The main apparatus of SWE was consisted of extractor (10 ml in volume; Thar Design Inc., USA), back pressure regulator (BPR; AKICO, Japan), high pressure pump (200 LC Pump, Perkin Elmer, Germany), and heater (memmert UN 55). There are removable threaded covers included stainless- steel filters (0.1 – 1 m) in both side of extractor. Beside that, the main apparatus of electrospraying was consisted of high voltage supply power (high voltage power supply model HGR30-20N, Japan), syringe pump (KD Scientific, USA), and a collector made of aluminium foil.

Subscritical water extraction process, 1 gr of raw material was loaded into extractor. The glass beads were put in the both sides of extractor inlet and outlet to prevent channeling. When the heater was installed with the extractor, the HPLC pump flowed water at 1 ml/min. flow rate into the extractor. And then, back pressure regulator (BPR) is used to control pressure according to the pressure gauge that monitors the pressure. Water was heated using a preheater and heater, to achieve the operating temperature. The temperature in inlet and outlet extractor were measured by thermocouple (T_1 and T_2) to ensure the temperature in the extractor according to desired temperature. Next the extract solution was cooled in the cooler, then pass through the filter, and collected in the collection vial. The extraction was carried out at temperature of 160ºC and pressure of 7 MPa for 3 hours. The schematic diagram of subcritical water extraction apparatus is shown in Figure 1. Schematic diagram of electrospraying apparatus is shown in Figure 2. The apparatus was consisted of syringe pump, power supply, and collector. In electrospraying process, firstly the solution extract was mixed with PVP (polyvinylpyrrolidone) sonically at the concentration of 4 and 6 % w/v. PVP is good for granulation, nanoparticle dispersant, and be a surface stabilizer. To measure the conductivity of the precursor solution using a conductometer. The humidity was monitored at that time. Next, put the precursor solution into syringe pump to spray. The tip of syringe has been charged with a high voltage generated by the power supply. The resulting particles would stick to the collector that has a different charge with the tip of syringe. The electrospraying was carried out at a distance between tips of 6-10 cm for 4 hours.

C. Analytical Method

Analysis of antioxidant activity in the extract was conducted using Genesys 10 UV-Vis Scanning Spectrophotometer (Thermo Fisher Scientific, Waltham, MA), allowing spectra between 190 nm to 1,100 nm. Liquid products were analyzed in quartz cuvette with 1 cm path length. Scanning electron microscopy (SEM) analysis was carried out to determine the particles morphology and the diameter of the particles produced. Beside that, the particles were analyzed by a TGA to determine material changes measured as a function of temperature.

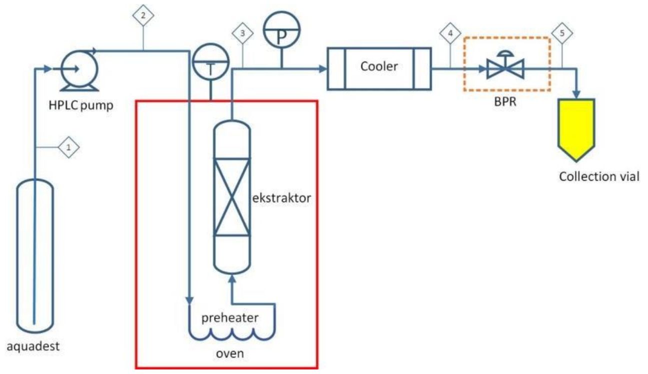

Figure 1. Apparatus for Hydrothermal Extraction

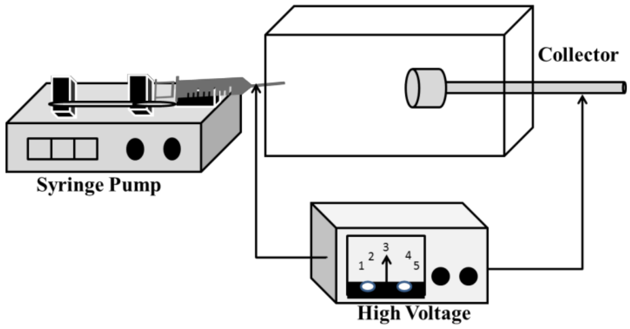

Figure 2. Schematic Diagram of Electrospraying Apparatus

D. Analytical Method

Analysis of antioxidant activity in the extract was conducted using Genesys 10 UV-Vis Scanning Spectrophotometer (Thermo Fisher Scientific, Waltham, MA), allowing spectra between 190 nm to 1,100 nm. Liquid products were analyzed in quartz cuvette with 1 cm path length. Scanning electron microscopy (SEM) analysis was carried out to determine the particles morphology and the diameter of the particles produced. Beside that, the particles were analyzed by a TGA to determine material changes measured as a function of temperature.

E. Antioxidant Efficiency

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method was an antioxidant assay based on electron-transfer. DPPH , a stable free radical , was reduced in the presence of an antioxidant molecule [13]. Simultaneous changes in purple to pale yellow occur when a DPPH solution was mixed with a substrate which acts as a hydrogen atom donor and as consequence the absorbance's decreased [14][15].DPPH assays could be easily and be quickly to evaluate antioxidants using spectrophotometry [13].

The antioxidant efficiency of extract was determined by adding 900 µL of particles diluted in aquadest with ratio 1:1 into 2 mL of 2 ppm DPPH in methanol solution. The wavelength used is 516 nm, where the absorbance was measured every minute until the absorbance value obtained was constant [16]. Percentage of the remaining DPPH was calculated with the following equation:

$$\% \text{ DPPH}_{rem} = 100\ x \frac{[DPPH]_{rem}}{[DPPH]_{t=0}} \qquad (1)$$

[DPPH]rem is the absorbance of the extract at a certain time, and $[DPPH]_{t=0}$ is the initial absorbance of DPPH. The efficiency of radical antioxidant was calculated by the following equation:

$$\text{AE} = \frac{1}{EC_{50} X\ t_{EC_{50}}} \qquad (2)$$

EC_{50} is the concentration of extract that caused 50% decrease in initial DPPH absorbance, and t_{EC50} is time needed to reach steady state at EC_{50} concentration

III. RESULTS AND DISCUSSION

The picture above shows the effect of various operating conditions on the mass of particles produced. Based on Figure 3, it can be seen that the mass of particles produced tends to increase according to the increase in voltage applied. The high voltage generated by the power supply results in an atomized precursor solution, where the solvent will diffuse into the air while the particles will stick to the collector. The higher the voltage applied, the greater the atomization that occurs. So that the mass of particles obtained is also getting bigger.

The higher the concentration of PVP added, the higher the mass of particles produced. This shows that PVP has the ability to bind phytochemical compounds in precursor solutions. In the electrospraying method, the concentration used should not be too high because it can produce fiber and cause blockages in the system

The distance between tips also plays an important role in the mass of particles produced. Based on figure 3, it can be seen that the greater the distance between the tip of the needle and the collector, the lower the mass produced. this is because the greater the distance between the two tips, the longer it takes for the particles to attach to the collector. Longer time is needed to reduce the amount of precursor solvents into the air. experimental operating conditions affect the value of antioxidant efficiency. The AE value is based on spectrophotometric measurements of changes in DPPH concentration produced by reactions with antioxidants. (DPPH) free radical scavenging method was a way to evaluate the antioxidant potential of a compound, extract or other biological source [17][18]. this was an easy method, applicable to measure overall antioxidant capacity. when the DPPH solution is mixed with substances that can donate hydrogen atoms, the absorption intensity will decrease during the incubation time. This results in a reduced DPPH form and loss of purple which turns pale yellow [19][20].

Figure 4 shows a decrease in the remaining DPPH (%) in the measurement of antioxidants. Based on Figure 4, it can be seen that the longer the incubation period of particles in DPPH solution, the decrease in DPPH% increases. This is because the particles produced contain phytochemical compounds extracted hydrothermal from eucheuma cottonii algae which act as antioxidants, which results in a reduction of DPPH compounds into non- radical compounds. This can be proven when the DPPH concentration reaches 50% of the initial DPPH concentration.

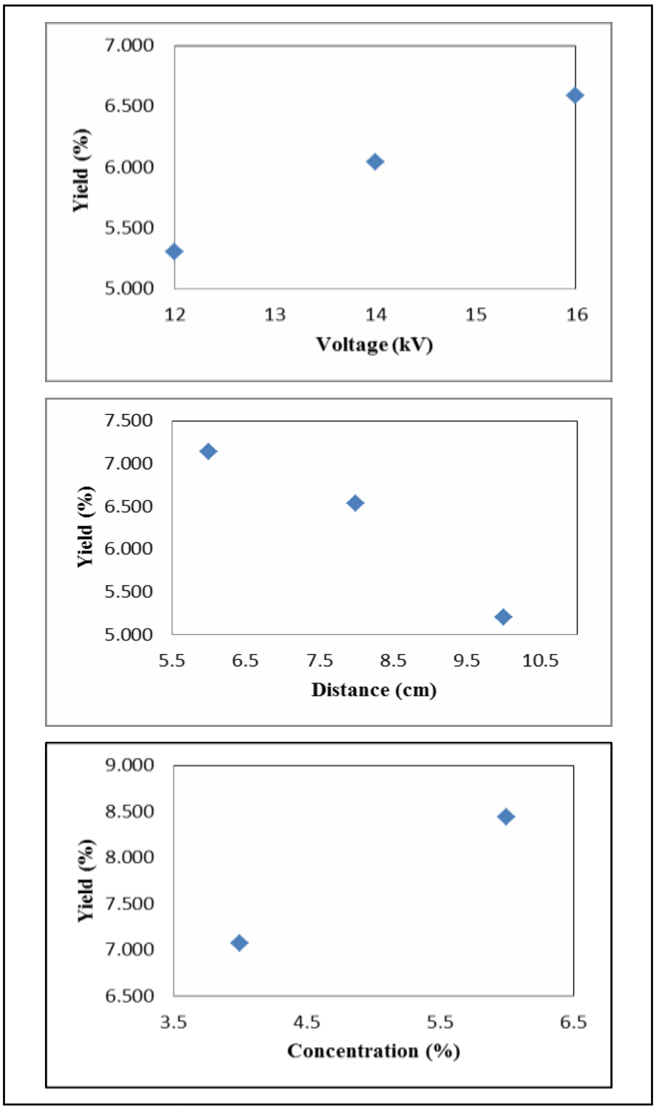

Figure 3. Mass of Particles Produced by Electrospraying at Various Conditions

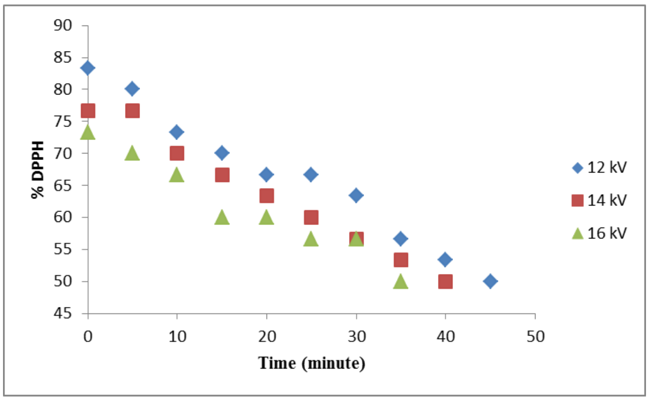

Figure 4. DPPH Remaining (%) in Time after The Addition of Particles

Table 1 shows the value of antioxidant efficiency of particles produced by electrospraying under various operating conditions. based on table 1 it can be seen that there are effects of operating conditions on the value of AE. AE values increase with increasing concentration. This happens because the greater the amount of PVP, the more phytochemical compounds contained. In addition, the increase in voltage and distance between tips also increases the AE value.

The greatest antioxidant efficiency value (AE) is obtained when the concentration of the precursor solution is 6%, the distance between tips is 10 cm and the given voltage is 16 kV. The value of AE obtained will be greater as the antioxidant concentration gets smaller. The shorter the time needed to reduce the remaining% DPPH, the greater the AE value obtained. the greater the AE value of particles, the greater the ability of particles to fight free radicals.

Table 1.
Antioxidant Efficieny of Particles at Various Operating Conditions

Operating Condition	AE
4% 12 kv 6cm	0.0093
4% 14 kv 6cm	0.0100
4% 16kv 6cm	0.0124
4% 12kv 8cm	0`.0109
4% 14kv 8cm	0.0119
4% 16kv 8cm	0.0256
4% 12kv 10cm	0.0171
4% 14kv 10cm	0.0200
4% 16kv 10cm	0.0333
6% 12kv 6 cm	0.0101
6% 14kv 6cm	0.0123
6% 16kv 6cm	0.0279
6% 12kv 8cm	0.0267
6% 14kv 8cm	0.0385
6% 16kv 8cm	0.0417
6% 12kv 10cm	0.0452
6% 14kv 10cm	0.0714
6% 16kv 10cm	0.1818

Particle morphology from SEM analysis can be seen in Figures 5 and 6. The particles produced by electrospraying are spherical. This is in accordance with the Twu et.al [21] study which states that the geometry of the polysaccharide produced by the spray drying process is a sphere with a smoother surface morphology. particle diameter size produced below 3µm.

Based on figure 5, the average particle diameters were 0,6964 ± 0,2087 µm, 0,6470 ± 0,2074 µm and 0,5349 ± 0,2358 µm for particle produced at distance between tips at 6cm, 8cm and 10 cm. It shows that the distance of the injection tip to the collector has an influence on the particle diameter because the difference in distance affects the evaporation speed and the settling time. The particle diameter decreases with increasing distance of the needle tip to the collector. that's because coloumb fission is divided into smaller particle particles as long as the particles are distributed from the needle to the collector when the distance increase[22]. Based on Figure 6, the size of the particle diameter is affected by the voltage applied. SEM images show that the microsphere is produced at a given voltage (12 and 16 kV) with a PVP concentration of 6%, a distance between 8 cm tips and a solution flow rate of 0.05 ml / hour. diameter size for 12 kV which is equal to 0,7268 ± 0,3499 µm. Whereas for a voltage of 14 kV equal to 0,7233 ± 0,3040 µm. and for 16 kV equal to 0,6241 ± 0,2789 µm. Particle size will be smaller with increasing voltage applied [23][24].Decreasing particle size occurs because of the increase in the strength of the magnetic field at a higher applied voltage [22].

Thermal gravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a sample is heated at a constant rate [25]. This TGA analysis is performed on each particle formation variable as well as on pure PVP components. In this TGA analysis, oxygen and nitrogen gas are used by c omparison (21:79). The maximum temperature used in this analysis is 500ºC with a temperature rise of 50ºC per minute

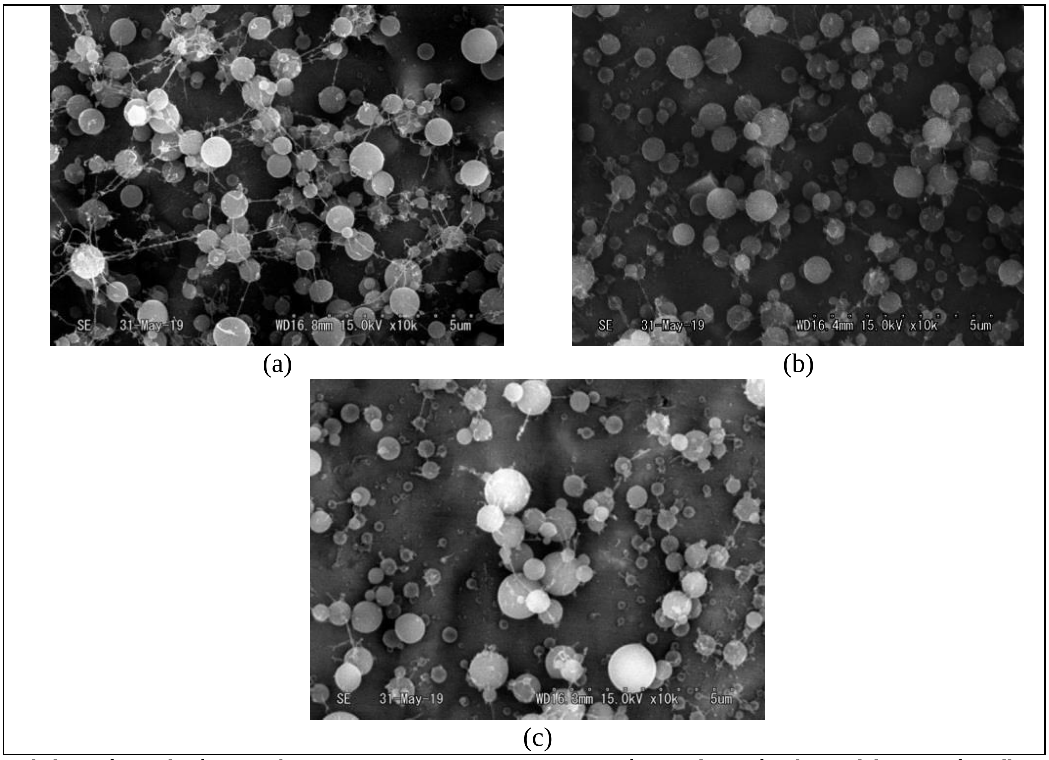

Figure 5. Morphology of particles from *Eucheuma cottonii* extract at concentration of 4%, voltage of 14 kV, and distance of needle tip to colector of (a) 6 cm; (b) 8cm; (c) 10cm

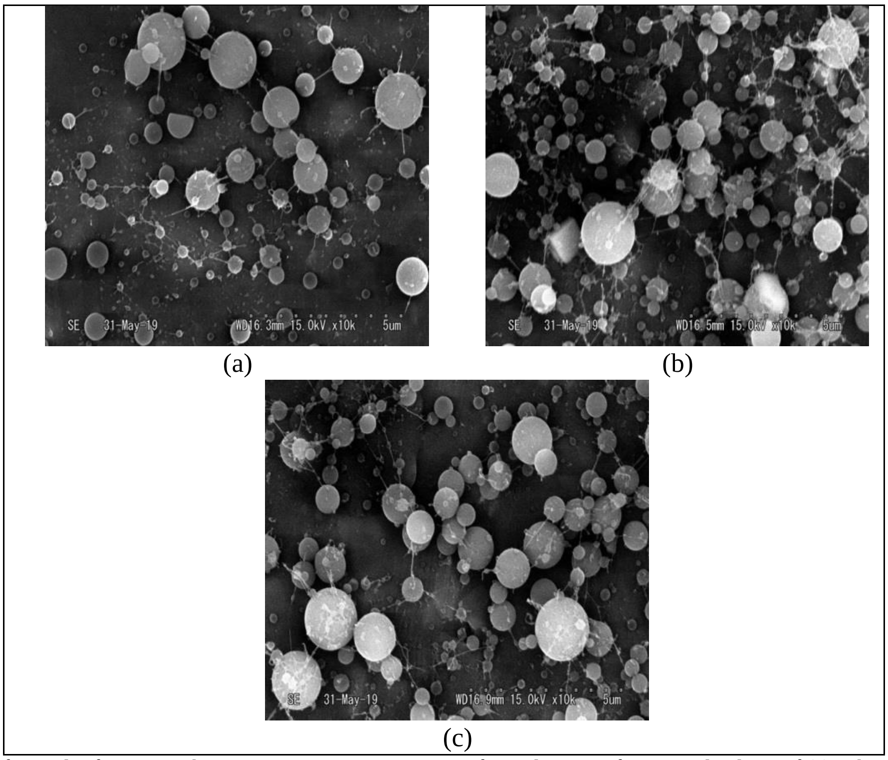

Figure 6. Morphology of particles from *Gracilaria* sp extract at concentration of 6%, distance of 8 cm, and voltage of (a)12 kV; (b)14kV; (c) 16 kV

Based on Figure 7,8,9, TGA analysis results show mass reduction that occurs in the temperature range of 417^{o}C to 500^{o}C. The sample mass continues to decline, this is due to the decomposition of PVP which begins to occur at a temperature of around 417^{o}C. The overall reduction in mass is due to an exothermic reaction that occurs in the temperature range of 417^{o}C – 500^{o}C. It can be seen that the peaks of particles that have different concentrations, voltage and distances of the needle tip to the collector have the same tendency. So that it can be seen, that the variable process of particle formation does not affect the material decomposition of these particles.

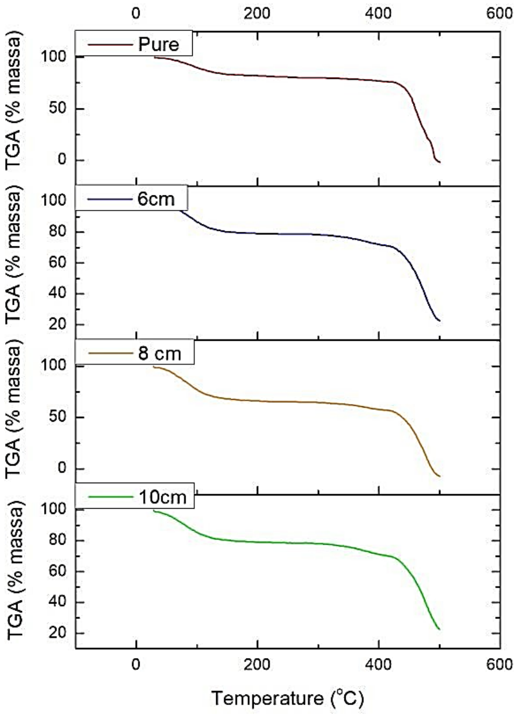

Figure 7. Comparative results of TGA analysis of Eucheuma cottonii algae which are processed under 12kv operating conditions and 6% concentration

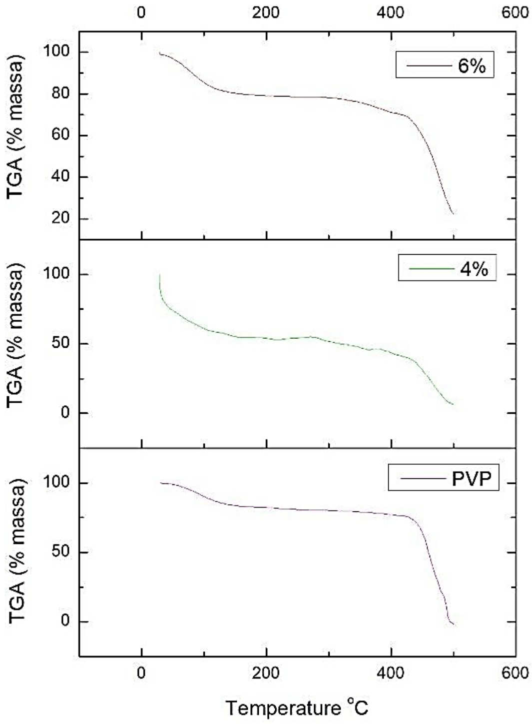

Figure 8. Comparative results of TGA analysis of Eucheuma cottonii algae which are processed under 12kV operating conditions and distance 1

I. CONCLUSION

When the distance greater than the tip of the needle to the collector and the voltage is applied, the size of the particle shrinks. The optimum condition for producing phytochemical particles is at 6% PVP concentration, the applied voltage is 16 kV, the tip distance and collector are 10 cm, and the solution flow rate is 0.05 mL / hour. Morphology of spherical particles with particle diameters below 3 µm. The value of antioxidant efficiency (AE) of particles showed that eucheuma cottonii extract contained in particles with antioxidant activity. The biggest AE value is 0.1818 minutes^{-1} obtained at a voltage of 16 kV and the distance of the tip to the collector is 10 cm particle size with operating conditions. The higher the voltage and the distance from the tip of the needle to the collector, the smaller the particle size.

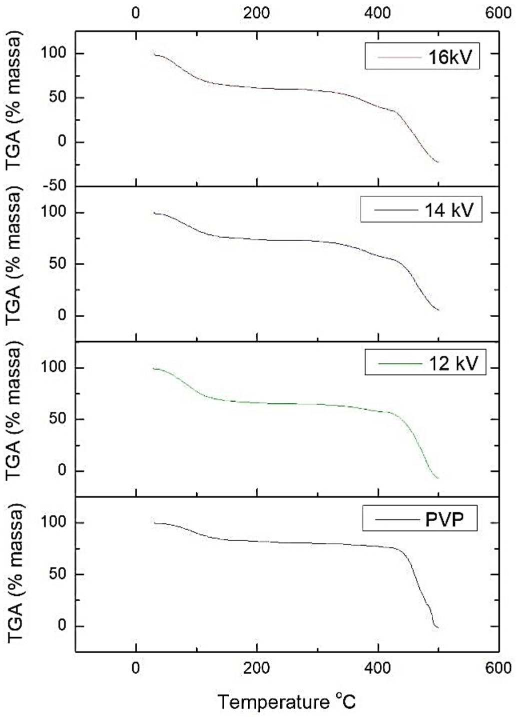

Figure 8. Comparative results of TGA analysis of Eucheuma cottonii algae which are processed under 8cm operating conditions and 6% concentration

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