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Characteristics and Free Radical Scavenging Activity of Zinc Oxide (ZnO) Nanoparticles Derived from Extract of Coriander (*Coriandrum sativum* L.)

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Abstract

Zinc oxide (ZnO) nanoparticles were recently studied for its free radical scavenging activity. The formation of ZnO nanoparticles by means of biological method was known to be a simpler and more environmental friendly method. One of the biological methods used for the formation of ZnO nanoparticles was by utilizing the phytochemicals that were present in a plant extract. The phytochemicals such as phenolic and flavonoid compound was able to reduce bulk metal Zinc into Zinc nanoparticles. Coriander (Coriandrum sativum L.) seeds and leaves were known to contain high amounts of phenolic compounds and flavonoid compounds. The aim of this study was to investigate the effect of different solvent polarities used to extract Coriander leaves and seeds towards the reducing power of plant extract and the free radical scavenging capacity of ZnO nanoparticles. The different solvents that were used to extract Coriander were deionized water, ethyl acetate, and hexane. The different solvent polarities affected the weight of the derived ZnO nanoparticles due to the different types of the extracted phenolic and flavonoid compounds. The different solvent polarities also affected the free radical scavenging capacity of ZnO nanoparticles. Ethyl acetate based extract of Coriander leaf resulted in the most weight of ZnO nanoparticles, 0.7907 g and the highest free radical scavenging capacity, 11.16%/mg ZnO nanoparticles. The free radical scavenging activity of ZnO nanoparticles decreased to 3.67%/mg ZnO nanoparticles after the process of calcination at 100°C and decreases to 0 %/mg ZnO nanoparticles after the process of calcination at 300 and 500°C. The diameter of ZnO particles increases from 128.1±33.5 nm to 552.3±61.1 nm after calcination.

Keywords: *calcination; free radical scavenging capacity; polarities; zinc oxide nanoparticles*

Abstrak

KARAKTERISTIK DAN AKTIVITAS PENANGKAL RADIKAL BEBAS NANOPARTIKEL SENG OKSIDA (ZnO) YANG DIBENTUK MENGGUNAKAN EKSTRAK KETUMBAR (*Coriandrum sativum* L.). *Nanopartikel Seng Oksida (ZnO) saat ini tengah diteliti sehubungan dengan kapasitasnya dalam menangkal radikal bebas. Metode biologis merupakan metode sederhana dan ramah lingkungan yang dapat digunakan pada pembentukan nanopartikel ZnO. Pemanfaatan senyawa fitokimia dari ekstrak tanaman merupakan salah satu metode biologis yang dapat digunakan pada pembentukan nanopartikel ZnO. Senyawa fitokimia seperti senyawa fenolik dan flavonoid dapat mereduksi logam Seng menjadi nanopartikel Seng. Biji dan daun dari tanaman ketumbar (Coriandrum* *sativum L.) telah diketahui memiliki kandungan senyawa fenolik dan flavonoid. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh perbedaan polaritas pelarut yang digunakan untuk ekstraksi biji dan daun ketumbar terhadap kapasitas reduksi ekstrak dan penangkal radikal bebas dari nanopartikel ZnO. Pelarut yang digunakan pada ekstraksi biji dan daun ketumbar adalah air deionisasi, etil asetat dan heksana. Perbedaan polaritas pelarut mempengaruhi berat nanopartikel ZnO yang diperoleh, hal ini disebabkan oleh perbedaan jenis senyawa fenolik dan flavonoid yang terekstrak. Perbedaan polaritas pelarut juga mempengaruhi kapasitas penangkal radikal bebas dari nanopartikel ZnO. Ekstrak etil asetat daun ketumbar menghasilkan nanopartikel ZnO dengan berat 0,7907 g dan aktivitas penangkal radikal bebas sebesar 11,16%/mg nanopartikel ZnO. Aktivitas penangkal radikal bebas nanopartikel ZnO menurun menjadi 3,67%/mg nanopartikel ZnO setelah dikalsinasi pada suhu 100ºC dan menurun menjadi 0%/mg nanopartikel ZnO setelah dikalsinasi pada suhu 300ºC, 500ºC. Diameter nanopartikel ZnO meningkat dari 128,1±33,5 nm menjadi 552,3±61,1 nm setelah dikalsinasi*

Kata kunci: *elektrokoagulasi, penurunan warna, penurunan TSS, laju degradasi COD, imbah tekstil*

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INTRODUCTION

Nanoparticles are being studied for its multifunctional properties in various fields such as health care, food, cosmetics, and environmental health (Kuppusamy *et al*., 2015; Vaseem and Umar, 2010). Recent studies also found that nanoparticles have unique chemical properties such as behaving as an antimicrobial agent and free radical scavenging agent (Das *et al*., 2013; Narmadha *et al*., 2013; Pai *et al*., 2014). The development of nanoparticles has known to increase the stability and the volume to surface ratio of the particles (Narmadha *et al*., 2013).

Nanoparticles are usually synthesized from metals such as silver, gold, platinum, copper, and zinc. There are several methods used for the synthesis of nanoparticles such as physical, chemical, and biological. Physical methods involves ball milling, thermal evaporate, and spray pyrolysis. Chemical methods involves electrodeposition, sol-gel process, and chemical solution deposition. Biological methods are the most ecofriendly method which utilizes resources such as plant extracts, bacteria, fungi, seaweed, and enzymes (Kuppusamy *et al*., 2015).

Plant extracts contain phytochemicals such as phenolic and flavonoid is able to reduce bulk metal into nanosized metal. A recent study investigated the formation of zinc oxide (ZnO) nanoparticles from Coriander (*Coriandrum sativum* L.) leaves extract. The study proves that *Coriandrum sativum* extract can be used to reduce salt of zinc to ZnO nanoparticles (Gnanasangeetha and Ravindran, 2013). Another study investigated the free radical scavenging activity of ZnO nanoparticles synthesized from *Citrus paradise* extract (Pai *et al*., 2014; Kumar *et al*., 2014).

Several studies stated that the formation of zinc oxide nanoparticles requires the process of calcination. The heat from calcination could cause changes in the size and morphology of ZnO nanoparticles. On previous studies, ZnO nanoparticles undergone the process of calcination at 100-600°C (Pai *et al*., 2014; Kumar *et al*., 2014). Therefore, in this study the effect of calcination towards particle size and shape were investigated.

In this experiment, Coriander (*Coriandrum sativum* L.) was extracted using solvents with different polarities; polar, less polar, and non-polar. Solvents with different polarities were used to extract phenolic and flavonoid compound that has different polarities. The aim of this experiment was to study the physical characteristics and free radical scavenging capacity of Zinc Oxide nanoparticles synthesized from Coriander (*Coriandrum sativum* L.) leaves and seeds extract.

MATERIALS AND METHODS

Materials

The leaf and seed of coriander (*Coriandrum sativum* L.). All the chemicals were of analytical grade. Deionized water, ethyl acetate, hexane, zinc acetate powder, sodium hydroxide (NaOH) solution, methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), folin-ciocalteu, gallic acid, quercetin, sodium carbonate (Na_2CO_3), and aluminum chloride ($AlCl_3$).

Coriander (*Coriandrum sativum* L.) Extraction

The coriander leaves and seeds were extracted according to method described previously (Pai *et al*., 2014; Kumar *et al*., 2014). Five grams of leaves/seeds of Coriander (*Coriandrum sativum* L.) were mixed with 100 mL solvent. The mixture was stirred for 3 h in magnetic stirrer. The mixture of leaves/seeds and solvent was filtered using Whatmann filter paper. The filtrate of the mixture was considered as the plant extract. A sample of the resulting extract was dried using oven method to obtain the dry matter of the extract. The filtrate was collected and the extract is stored at 4°C. Both of leaves extract and seeds extract were analyzed for its total phenolic content and total flavonoid content

Reduction Process

50 mL of 0.6 M zinc acetate (aq) solution was mixed with 0.5 g dry matter of Coriander (*Coriandrum sativum* L.) extract. After the solution was mixed, the

pH was adjusted to pH 8 using 1 M NaOH. The mixture was stirred for 3 h at 75-80°C until a color change was observed (yellowish green to light yellow). The resulting ZnO nanoparticles were separated by centrifugation at 5000 rpm for 10 minutes, then washed with deionized water three times and dried in a hot air oven at 60°C overnight. The ZnO nanoparticles powder was analyzed for its free radical scavenging activity. The control used for this analysis was aqueous zinc acetate solution (Kumar *et al*., 2014).

Free Radical Scavenging Activity

In this research, the free radical scavenging activity (RSA) of the ZnO nanoparticles powder was measured using DPPH method. ZnO nanoparticles powder was dissolved using distilled water until a concentration of 50 mg/mL. The mixture was mixed using vortex for 5 minutes. The precipitate of the solution was dried in the oven and weighed. 1 mL of the supernatant was mixed with 1.5 mL of 0.2 mM of DPPH solution in absolute methanol. The mixture was shaken vigorously and allowed to stand in room temperature for 30 minutes in the dark. The concentration of ZnO nanoparticles powder was measured as the amount of ZnO nanoparticles powder that dissolves in water. The absorbance of the mixture was measured using a spectrophotometer at a wavelength of 517 nm. A mixture of methanol and DPPH solution was used as blank instead of the ZnO nanoparticles solution. The free radical scavenging activity was measured as % RSA/mg dissolved sample (Kumar *et al*., 2014).

Calcination

The obtained powder from the reduction process was weighed and placed inside a crucible. The crucible is then put into a muffle furnace and heated to a temperature of 100, 300, and 500ºC for 3 h. After calcination process, the powder is stored. The ZnO nanoparticles that were analyzed for its free radical scavenging activity. The ZnO nanoparticles with the highest free radical scavenging activity will be analyzed for its size and shape by Scanning Electron Microscopy (SEM) and Particle Size Analyzer (PSA) (Parra *et al*., 2014).

Experimental Design

The experimental design used in this experiment was completely randomized single-factorial design with three variables and two replications. The variable used was the type of solvent. All data was processed using SPSS software, version 20. One-way ANOVA and Independent sample t-test was used to test the significant (α = 0.05) mean difference among treatments while Duncan test was used to compare differences among means. The different solvents used were deionized water, ethyl acetate, and hexane.

Total Phenolic Content

Total phenolic content analysis was performed towards the Coriander (*Coriandrum sativum* L.) plant extract according to previous method (Handayani *et al*., 2013). To assess the total phenolic content, 1 mL of the Coriander plant extract was put into 10 mL test tube. 5 mL of Folin Ciocalteu 10% solvent and 4 mL of sodium carbonate 7.5% were added to the test tube and mixed thoroughly. The mixture was settled down for one-hour and the absorbance is checked using a spectrophotometer at 760 nm. The total phenolic content was calculated based on the absorbance level using linear regression curve with using gallic acid as control. The result was expressed as equivalent of gallic acid/100 grams Coriander plant extract.

Total Flavonoid Content

Two mL of plant extract was mixed with 2 mL of 2% $AlCl_3$ solution dissolved in Methanol. The mixture was placed in dark room for 10 minutes. After 10 minutes the absorbance of the mixture was measured at 415 nm. 1 ml of methanol and 1 ml of $AlCl_3$ 2% was used as blank. The absorbance of the sample was then compared to the standard curve made from quercetin and the result was expressed in milligram quercetin equivalent (QE)/gram of extract (Lamien-Meda *et al*., 2014).

Scanning Electron Microscopy (SEM)

The powder sample of the ZnO nanoparticles solution was sonicated with distilled water. A small drop of sample was placed on glass slide allowed to dry and place inside Jeol JSM 6510 scanning electron microscope to be processed.

Particle Size Analyzer (PSA)

The particle size analyzer was conducted using PSA300 static image analysis system, which includes a microscope and a digital camera, which collect images of particles as the slide containing the sample was scanned.

RESULTS AND DISCUSSION

Plant Extract Properties

The plant extract was dried in the oven in order to obtain the amount of dry matter in the extract. This was done in order to ensure that the phytochemicals used for reduction of zinc acetate into ZnO nanoparticles were in the same amount at all reactions. The amount of dry matter in the extracts were shown in Table 1.

Table 1. Dry matter of Coriander (*Coriandrum sativum* L.) leaves and seeds extract

Leaves		Seeds	
Type Of Solvents	Dry matter (g)	Type Of Solvents	Dry matter (g)
Water	25.66	Water	5.65
Ethyl Acetate	0.50	Ethyl Acetate	3.60
Hexane	0.85	Hexane	3.05

Total Phenolic Content

Table 2 show the amount of phenolic compounds present in leaves and seeds extracts. It can be concluded that the leaves of Coriander (*Coriandrum sativum* L.) contain the highest phenolic compound when extracted using ethyl acetate. It can be deduced that the leaves of Coriander contains mostly semi-polar phenolic compounds, followed by non polar phenolic compounds, then polar phenolic compounds. The seeds of Coriander contains mostly semi-polar phenolic compounds, followed by non-polar compounds, the polar compounds.

According to statistical analysis, by using semi-polar solvent, it gives a significant difference in the amount of phenolic compounds extracted in both leaves and seeds of Coriander (*Coriandrum sativum* L.) than polar and non-polar solvents ($p \leq 0.05$). Ethyl acetate was the highest then followed by hexane, then followed water.

Table 2. Total phenolic content of Coriander (*Coriandrum sativum* L.) leaves and seeds extract

Leaves		Seeds	
Type Of Solvents	Total Phenolic (mg GAE/g sample)	Type Of Solvents	Total Phenolic (mg GAE/g sample)
Water	$0.7022^a \pm 0.0222$	Water	$2.4955^a \pm 0.1129$
Ethyl Acetate	$51.5717^c \pm 2.1478$	Ethyl Acetate	$7.2754^c \pm 0.3370$
Hexane	$11.5178^b \pm 0.4260$	Hexane	$4.9533^b \pm 0.1907$

Note: different superscript notation that presented above every coloumn showed significant difference ($p<0.05$).

Total Flavonoid Content

Based on statistical analysis, the use of solvents with different polarities causes a significant difference in the amount of total flavonoid compound extracted in the leaves and seeds of Coriander extract (*Coriandrum sativum* L.) ($p \leq 0.05$). Semi-polar solvent being the highest, followed by polar, then non-polar ($p \leq 0.05$).

Table 3. Total flavonoid content of Coriander (*Coriandrum sativum* L.) leaves and seeds extract

Leaves		Seeds	
Type Of Solvents	Total Flavonoid (mg QE/g sample)	Type Of Solvents	Total Flavonoid (mg QE/g sample)
Water	$1.3786^a \pm 0.1059$	Water	$7.0939^b \pm 0.3476$
Ethyl Acetate	$41.9026^c \pm 3.1051$	Ethyl Acetate	$16.6579^c \pm 0.7145$
Hexane	$10.7368^b \pm 2.8370$	Hexane	$0.6763^a \pm 0.0234$

Note: different superscript notation that presented above every coloumn showed significant difference ($p < 0.05$).

Reduction of Zinc Oxide (ZnO) Nanoparticles

The process of reduction of Zinc Acetate into ZnO nanoparticles involves the reaction of the phytochemicals (phenolics and flavonoids) in Coriander (*Coriandrum sativum* L.) plant extract with zinc acetate. The phytochemicals act as a reducing agent for zinc acetate. The reaction of zinc acetate and plant phytochemicals resulted in Zn (flavonoids, phenolics) complex is dried in a 60ºC oven. During this drying process, the Zn (flavonoids, phenolics) complex is converted in to ZnO nanoparticles (Das *et al*., 2013; Malik *et al*., 2014; Vaseem and Umar, 2010; Kumar *et al*., 2014).

Weight of Zinc Oxide (ZnO) Nanoparticles

Table 4 shows the weight of ZnO nanoparticles synthesized using seeds and leaf extract of Coriander (*Coriandrum sativum* L.). Ethyl acetate base extract of the leaves and seeds of Coriander has the highest reducing power among other extracts ($p \leq 0.05$). However, based on t-test analysis between the reducing power of ethyl acetate based leaf extract and ethyl acetate based seed extract showed that ethyl acetate based leaf extract is not significantly higher than ethyl acetate based seed extract ($p \geq 0.05$).

Table 4. Weight of resulting ZnO nanoparticles synthesized using Coriander (*Coriandrum sativum* L.) leaves and seeds extract

Leaves		Seeds	
Type Of Solvents	Weight of ZnONPs (mg)	Type Of Solvents	Weight of ZnONPs (mg)
Water	$0.5401^a \pm 0.1648$	Water	$0.3960^a \pm 0.0880$
Ethyl Acetate	$0.7907^b \pm 0.800$	Ethyl Acetate	$0.7266^b \pm 0.1165$
Hexane	$0.3817^a \pm 0.0732$	Hexane	$0.4390^a \pm 0.0403$

Note: different superscript notation that presented above every coloumn showed significant difference ($p<0.05$).

The use of solvents with different polarities causes a significant difference in the weight of ZnO nanoparticles ($p \leq 0.05$). The weight of ZnO nanoparticles derived using both ethyl acetate based extracts of leaves and seeds of Coriander correlates with the phenolic and the flavonoid content of the respective extracts. This result explains that semi-polar phenolic and flavonoid compound probably is the most suitable compound that can be used to produce ZnO nanoparticles. One example of a semi-polar phytochemical present in Coriander leaves and seeds were p-coumaroylquinic acid and rutin (Muñiz-Márquez *et al*., 2013; Rajeshwari and Andallu, 2011).

Free Radical Scavenging

The free radical scavenging activity of ZnO nanoparticles was measured as the %radical scavenging activity (RSA)/mg ZnO nanoparticles. From Table 5, it can be deduced that the synthesized ZnO nanoparticles posses free radical scavenging activity. Zinc acetate or the bulk zinc metal did not posses any free radical scavenging activity. It can be concluded that ZnO nanoparticles could act as a free radical scavenger.

Thus the results were in correlation with the previous study (Kumar *et al.*, 2014), which states that ZnO nanoparticles synthesized using the reduction method by plant extract would posses free radical scavenging activity. In conclusion, it can be deduced that ZnO nanoparticles that is reduced using ethyl acetate based leaf extract of Coriander (*Coriandrum sativum* L.) showed the highest free radical scavenging activity, followed by water based, then hexane based ($p \leq 0.05$).

Table 5. Free radical scavenging activity of ZnO nanoparticles from Coriander (*Coriandrum sativum* L.) leaves and seeds extract

Leaves		Seeds	
Type Of Solvents	Free Radical Scavenging Activity (%/mg ZnO)	Type Of Solvents	Free Radical Scavenging Activity (%/mg ZnO)
Water	$7.50^{b} \pm 1.63$	Water	$1.65^{a} \pm 0.66$
Ethyl Acetate	$11.16^{c} \pm 3.28$	Ethyl Acetate	$1.35^{a} \pm 0.57$
Hexane	$1.61^{a} \pm 0.58$	Hexane	$1.10^{a} \pm 0.53$

Note: different superscript notation that presented above every coloumn showed significant difference ($p<0.05$).

The free radical scavenging capacity of ZnO nanoparticles may be due to the transfer of electron density located at oxygen to the odd electron located at nitrogen atom in DPPH (Das *et al*., 2013). However, the ZnO nanoparticles that were derived using Coriander seeds extract of different solvent polarities showed that there is no significant difference in the free radical scavenging activity of the ZnO nanoparticles ($p \geq 0.05$). With values ranging from 1.1%/mg ZnO nanoparticles (Hexane based) to 1.65%/mg ZnO nanoparticles (water based). Based on t-test results, the free radical activity of ZnO nanoparticles derived using the leaves of Coriander (*Coriandrum sativum* L.) is significantly higher than the free radical scavenging activity of ZnO nanoparticles derived using the ethyl acetate based extract of the seeds of Coriander ($p \leq 0.05$). The result from this experiment correlates with the previous study (Kumar *et al*., 2014), which utilizes water based extract of Citrus paradisi peel to produce ZnO nanoparticles. The resulting ZnO nanoparticles is able to donate electron to DPPH causing it to change color from purple to yellow, thus the ZnO nanoparticles were known to posses free radical scavenging activity

Calcination

Table 6 shows the free radical scavenging activity of ZnO nanoparticles derived from ethyl acetate based Coriander leaf extract after calcination at 100, 300, and 500°C. It can be seen that the free radical scavenging activity of ZnO nanoparticles decreases after the process of calcination. The ZnO nanoparticles that did not undergone the process of calcination showed the highest free radical scavenging activity at 11.16%/mg ZnO nanoparticles, then followed by the ZnO nanoparticles that undergone the process of calcination at 100ºC which is 3.47%/mg ZnO nanoparticles.

The ZnO nanoparticles that undergone the process of calcination at 300 and 500 ºC showed no free radical scavenging activity. Based on the statistical analysis, it can be concluded that calcination temperature significantly affects the free radical scavenging activity of ZnO nanoparticles ($p \leq 0.05$).

Table 6. Free radical scavenging activity of ZnO nanoparticles derived from Ethyl Acetate based Coriander (*Coriandrum sativum* L.) leaves extract after calcination at different temperatures

Temperature (ºC)	Free Radical Scavenging Activity (%/mg ZnO nanoparticles)
0	$11.16^{c} \pm 0.57$
100	$3.47^{b} \pm 2.09$
300	$0.00^{a} \pm 0.00$
500	$0.00^{a} \pm 0.00$

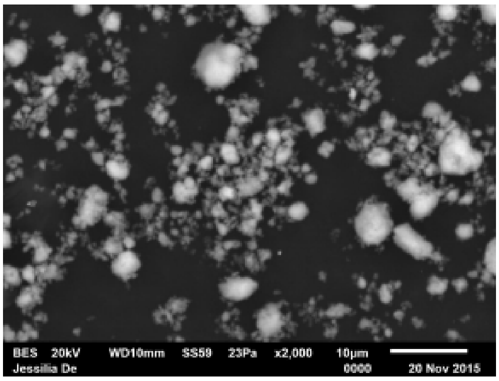
Note: different superscript notation indicates a significant difference between samples ($p<0.05$)

The free radical scavenging activity of ZnO nanoparticles is due to the interaction between plant phytochemical and Zn^{2+} ion. This interaction causes a release of H^{+} to free radicals, thus causing the free radicals to be reduced. The heat from calcination could cause a disruption between those interactions, thus causing the ZnO nanoparticles to lose its free radical scavenging activity.

Size and Morphology

Figure 1 show the scanning electron micrograph of ZnO nanoparticless synthesized using Coriander (*Coriandrum sativum* L.) leaves ethyl acetate based extract after and before calcination. Both figure shows a particle like morphology.The diameter of ZnO nanoparticles before calcination was 128.1± 33.5 nm. This particle can be concluded as a nanoparticle because its diameter is still in the nanometer scale. Thus, it can be concluded that the reduction of bulk zinc by ethyl acetate based extract of the leaves of Coriander is able to produce zinc oxide nanoparticles.

According to the Particles Size Analyzer, the diameter of ZnO nanoparticles before calcination is 128.1±33.5 nm while after calcination is 552.3±61.1 nm. Calcination will cause nanoparticles to agglomerate, thus resulting in a larger particle size. The heat from calcination causes an increase particle nucleation rate which causes particle to agglomerate, thus causing an increase in particle size (Malik *et al*., 2014; Vaseem and Umar, 2010).



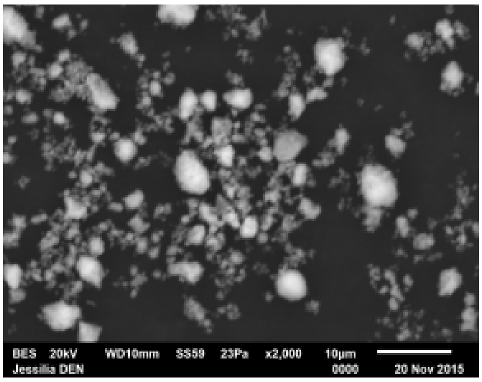


Figure 1. Scanning Electron Microscopy (SEM) images of the ZnO nanoparticles synthesized using Coriander (*Coriandrum sativum* L.) leaves ethyl acetate based extact before calcination (a) and after calcination (b).

CONCLUSION

Leaves and seeds of Coriander (*Coriandrum sativum* L.) can be used as a reducing agent to reduce bulk zinc acetate into ZnO nanoparticles. The ethyl acetate based leaf extract of Coriander showed the highest reducing power which can be represented by most weight of ZnO nanoparticles, 0.7266 grams. It can be concluded that semi-polar solvents such as ethyl acetate is able to extract the most phenolic compound 101.9266 mg GAE/g sample and flavonoid compound 43.3791 mg QE/g sample. The ZnO nanoparticles derived using ethyl acetate based leaf extract showed the highest free radical followed by water then hexane. However, there was no significant effect of different solvent polarities used to extract the seeds of Coriander to the free radical scavenging activity of the ZnO nanoparticles. Thus it can be concluded that the leaves of Coriander extracted using semi-polar solvents produced ZnO nanoparticles with the highest free radical scavenging activity, 11.16%. The free radical scavenging activity of ZnO nanoparticles decreases from 11.6%/mg ZnO nanoparticles to 3.47%/mg ZnO nanoparticles after the process of calcination at 100°C. Moreover, the free radical scavenging activity decreases to 0%/mg ZnO nanoparticles after the process of calcination at 300 and 500°C. The size of nanoparticle increases from 128.1±33.5 nm to 552.3±61.1 nm after calcination.

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