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SOLID DISPERSION OF USNIC ACID – PVP K30 AND EVALUATION OF ANTIOXIDANT ACTIVITY

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ABSTRACT

Usnic acid, a compound produced by Usnea sp, has been reported for anti-oxidant activity. However, the use of usnic acid is still finite due to poor solubility in water. One of processes to increase the solubility is by preparing into solid dispersion using hydrophilic polymer. The aims of this study were to prepare usnic acid in solid dispersion using poly-vinyl-pyrrolidone (PVP) K30 and evaluate its antioxidant activity. Solid dispersions were prepared using spray and freeze drying method at ratio of usnic acid:PVP of 1:1 and 1:2 (w/w). Physical mixture at the same ratio was prepared as comparison. The morphology of solid dispersion, which was depicted in scanning electron microscopy (SEM) analysis, showed rod crystal for usnic acid; sphere particles for PVP K30 and spray dried powder; porous particles for freeze dried powder; and usnic acid embedded on the surface of PVP K-30 for physical mixture. The amount of usnic acid dissolved in water was determined using a UV-Vis spectrophotometer. Freeze dried usnic acid-PVP at ratio 1:2 showed the highest solubility which was about 20-fold compared to intact usnic acid. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and gallic acid was used as the reference. The result of antioxidant activity test showed the inhibition concentration 50% (IC_{50}) were 12.471, 80.242 and 63.867 µg/ml for gallic acid, intact usnic acid and freeze dried usnic acid at ratio 1:2, respectively. In conclusion, solid dispersion is able to increase the solubility of usnic acid and the antioxidant activity is in accordance with the solubility result.

Keywords: Usnic acid, solid dispersion, PVP K30, antioxidant activity, freeze drying, spray drying.

INTRODUCTION

Lichens, symbiosis of fungi and algae, have ability to survive in extreme circumstances particularly when they are exposed to ultra-violet radiation that can scavenge radicals in order to protect the thalli[1]. This ability leads to potential activity of lichens as natural pro-oxidant and anti-oxidant. One of most major secondary metabolites produced by lichen species, such as Usnea sp, is usnic acid. Usnic acid has been reported for its pharmacological activities as antibacterial, antiviral, anti-protozoa, anti-inflammation, antipyretic, anti-proliferation, anti-tumor, and antioxidant[2,3]. However, these remarkable activities are still limited in used due to the psychochemical properties of usnic acid, that is poorly soluble in water (<0.01 g/100ml)[4].

Many studies have discussed some methods to improve the solubility of usnic acid including microencapsulation using PLGA[5], inclusion complex using cyxclodextrine[6], micellar solution[7], and milling process[8]. Among the methods mentioned above, solid dispersion is one the most popular methods to modify the psychochemical property active ingredient by

dispersing the drug in either sugar, hydrophilic polymer or surfactant[9]. Our previous study has elaborated preparation and characterization of usnic acid in HPMC[10].

The aim of this study was to prepare solid dispersion of usnic acid by two different methods, spray and freeze drying using PVP K-30, in order to increase its solubility and to evaluate the antioxidant activity. The antioxidant activity was evaluated by using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method, a relatively fast and easy technique to determine activity by spectrophotometry[11].

EXPERIMENITAL

Materials:

Usnic acid was isolated from Usnea sp as described in previous work[8], PVP K-30 (Shin-Etsu Chemical, Japan), DPPH (Sigma Aldrich, USA), gallic acid (Sigma Aldrich, USA), ethanol p.a (Merck, Germany), and distilled water.

Methods

Preparation of solid dispersion by spray drying method

Usnic acid and PVP K30 at ratio 1:1 and 1:2 (w/w) were dispersed in 100 ml distilled water and the mixture was stirred prior and during the drying process. Spray dryer (BUCHI Mini spray dryer B-290, Switzerland) was set as follow: inlet temperature 120°C and outlet temperature 60°C, flow 35m^3/hour, and diameter of nozzle 0.7 mm. The dried powder was collected from the particle collection chamber and kept in a desiccator.

Preparation of solid dispersion by freeze drying method

Usnic acid and PVP K-30 as the same ratio of spray drying method were mixed and homogenized prior to drying process. Liquid nitrogen was used to freeze the mixture. Freeze dryer (Christ Alpha 1-2 LD Plus, Germany) was set for primary drying at temperature -20°C for 12 hours and continued to secondary drying at temperature 20°C for 12 hours. The dried powder was collected from the flask and kept in a desiccator.

Preparation of physical mixture

Usnic acid and PVP K-30 as the same amount of solid dispersion were mixed physically in a jar until the mixture was homogenous and the mixed powder was then kept in a desiccator.

Scanning Electron Microscope (SEM) analysis

The morphology analysis of intact usnic acid, intact PVP K-30, physical mixture and solid dispersion was done using a SEM device (HITACHI type S-3400N, Japan) with the following condition: voltage 10 kV and current 12 mA. A small amount of each sample was placed in a sample holder and sample was observed at several magnifications.

Solubility test

The solubility of intact usnic acid, physical mixture and solid dispersion was determined in free carbo-dioxide water. An excessive amount of sample was dispersed in water and shake in a water bath shaker at room temperature for 24 hours. The amount of usnic acid dissolved in water was measured spectrophotometry using UV-spectrophotometer (Shimadzu UV-1700, Japan) at maximum wavelength of usnic acid in water (283nm). All the samples were filtered using Whatman filter paper before measurement. All the solubility experiments were done triplicate.

Free radical scavenging activity

Free radical scavenging activity was determined using DPPH method. Gallic acid, which is known to have a strong antioxidant activity, was used as reference standard. 0.1mM DPPH was added into a series concentration of gallic acid solution, intact usnic acid, PVP K-30, physical mixture and solid dispersions. Each solution was incubated at room temperature 20°C for 30 minutes. The absorbance of samples and reference standard was done at maximum length of DPPH in ethanol using UV-spectrophotometer (Shimadzu UV-1700, Japan) at 515.5 nm. Scavenging activity was measured by following % inhibition equation:

$$\%\ inhibition = \frac{(Abs\ DPPH\text{-}Abs\ Sample)}{Abs\ DPPH}\ x\ 100\%$$

Determination of IC_{50} value

IC_{50} value is defined as the minimum concentration of samples to inhibit 50% DPPH radical which was calculated by linear regression of samples concentrations to % inhibition. The IC_{50} value correlates to the strength antioxidant activity.

Data analysis

The data result of solubility and antioxidant activity was analyzed statistically by one way ANOVA with significance 95% using SPSS 22.0.

RESULTS AND DISCUSSION

Solid dispersion is one of many methods that has been used extensively to increase the solubility of poorly water soluble drugs which can be prepared with hydrophilic polymer, such as PVP K-30[12,13,14]. In our previous study, solid dispersion of usnic acid with HPMC has increased the solubility of usnic acid about 5 times[10]. In this study, PVP K-30 is used which is anticipated to improve the solubility of usnic acid better than HMPC.

One of most necessary characterization of solid dispersion is scanning electron microscopy (SEM). SEM is a prominent analysis to identify and observe the morphological changes of powder, particularly after modified by several processes. As can be seen in Figure 1(a), usnic acid crystal looks like rod and PVP K-30 in Fig 1(b) has spherical shape. The physical mixture (ratio 1:2), Fig 1(c) shows that usnic acid was embedded on the surface of PVP-K30. The spray dried usnic acid – PVP K-30 (ratio 1:2) depicts the spherical particles while freeze dried (ratio 1:2) shows an irregular shape as see Fig 1 (d) and (e). The differences on the morphology corresponded to the process utilized. The physical mixture applied lower energy compared to solid dispersions, which caused no change in the morphology of both usnic acid and PVP K-30. On contrary, solid dispersion by spray and freeze drying techniques applied higher energy that contributed in morphology changes. Spray drying process used nozzle which yields on spherical particles during drying process caused by heat energy[12]. Meanwhile, freeze drying applied sublimation process on the drying process resulted irregular shape and porous particles[15]. These changes are expected to influence the solubility of usnic acid.

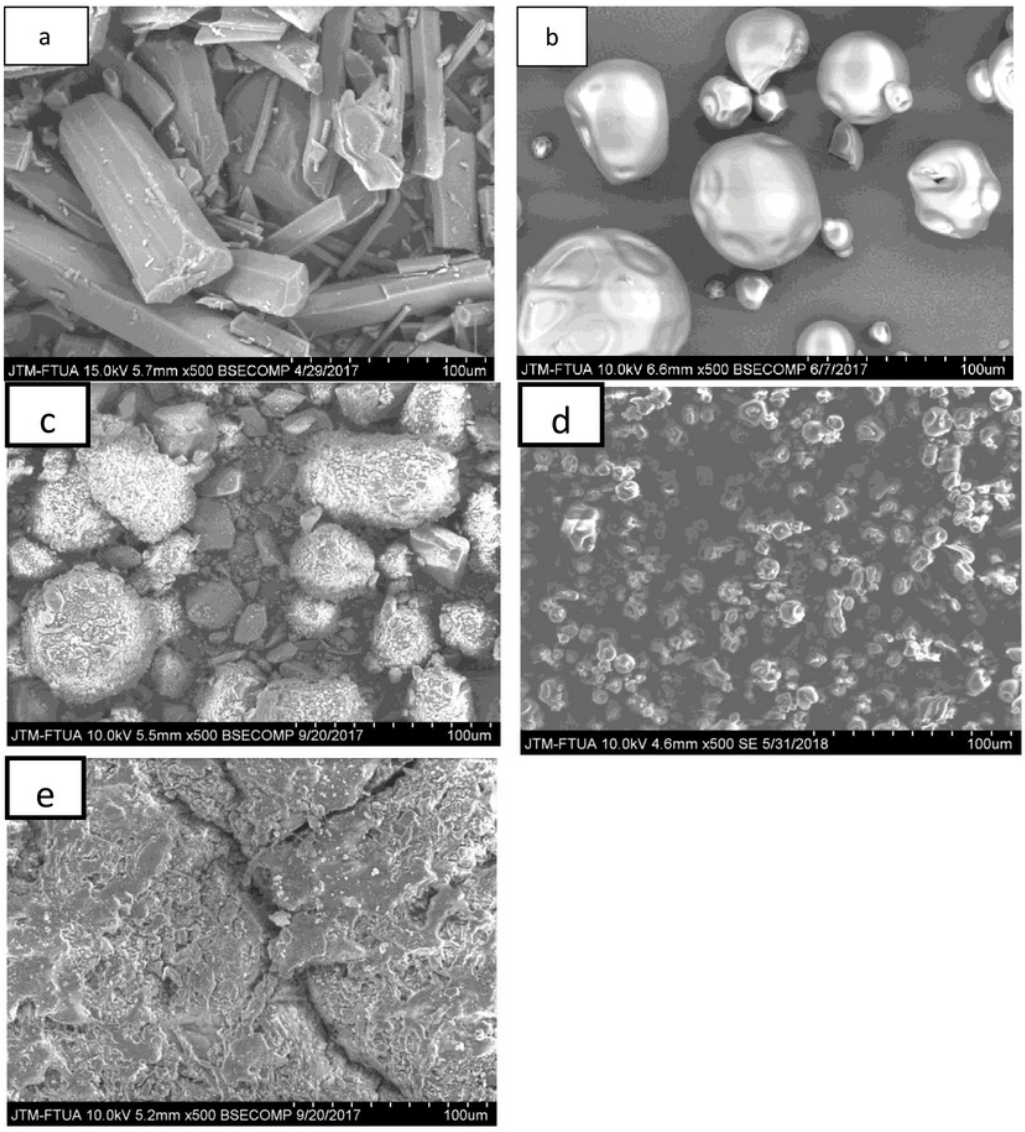

Figure 1. SEM photos of (a) usnic acid, (b) PVP K-30, (c) physical mixture, (d) solid dispersion by spray dried, and (e) solid dispersion by freeze dried.

The result of solubility test is shown in Table 1. The solubility of usnic acid significantly increased after mixed with PVP K-30 ($p<0.05$). Moreover, the highest solubility was performed by freeze dried at ratio 1:2. The impact of PVP K-30 in increasing the solubility has been discussed in many studies[12,13,14]. PVP K-30 is a hydrophilic polymer that easily dispersed and dissolved in water[16]. The amount of PVP K-30 also influences the solubility, where ratio 1:2 for all prepared formulas had higher solubility compared to ratio 1:1. Therefore, the solubility of usnic acid in physical mixture also improved significantly ($p<0.05$). In addition, the changes in morphology of usnic acid in solid dispersion preparation also affects the solubility. The spray dried particles had spherical shape and smaller particle size than intact usnic acid which caused greater surface area[12]. Furthermore, freeze dried

powder had small and big porous that also impact on the solubility due to more contact in water [15].

Table 1. The solubility test result

Usnic Acid (UA) (µg/ml)	Ratio UA: PVP K-30	Physical Mixture (µg/ml)	Spray dried (µg/ml)	Freeze dried (µg/ml)
55 ± 0.02	1:1	224 ± 0.24	376 ±0.47	929 ± 0.14
	1:2	277 ± 0.11	577 ±0.09	1,120 ± 0.06

The improvement in solubility of usnic acid is also anticipated in anti-oxidant activity. Antioxidants are electron-giving compounds that have ability in inactivating the oxidation reaction by radicals through preventing formation of radicals or neutralizing existed radicals such as DPPH[17]. DPPH is a purple solution in ethanol, when added a substance that has antioxidant properties, it will change into yellowish solution. This color change can be qualitative and quantitative parameters for determining anti-oxidant activity. The scavenging activity of gallic acid, usnic acid, PVP K-30, physical mixture (PM), spray dried (SD) and freeze dried (FD) at different concentration is calculated by % inhibition as seen in Table 2.

Table 2. % inhibition of each sample at different concentrations

Gallic acid		% inhibition								
Conc. (ppm)	% inhibition	Conc. (ppm)	PVP	Usnic acid	PM (1:1)	PM (1:2)	SD (1:1)	SD (1:2)	FD (1:1)	FD (1:2)
4	10.52	10	0	22.41	22.241	22.92	26.65	28.18	26.99	28.69
5	13.24	30	0	30.89	30.73	31.40	35.48	36.67	35.82	37.18
6	18.50	50	0	38.53	38.53	38.87	42.61	44.14	42.954	44.652
8	28.86	70	0	45.33	46.01	46.34	50.424	51.61	50.764	52.122
10	38.53	90	0	54.15	53.82	54.49	58.404	59.76	58.744	60.272
IC_{50} µg/ml	12.42	IC_{50} µg/ml	0	80.24	79.91	78.68	68.57	68.17	67.70	63.87

The ability or the antioxidant strength of a substance is determined by the value of inhibition concentration (IC_{50}), which defines as the concentration of compound which can inhibit DPPH activity by 50%. The lower the IC_{50} value of a compound, the stronger the antioxidant properties[17]. According to IC_{50} value, antioxidant activity of a compound is classified as strong activity if IC_{50} 10-50 ppm; moderate activity if IC_{50} >50-100 ppm; weak activity if IC_{50} >100-250 ppm; and no activity if IC_{50}150-200 ppm[18]. The IC_{50} value of usnic acid, physical mixture and solid dispersions was in the range 50-100 ppm and included as moderate antioxidant group, while galllic acid had a strong activity, as seen in Table 2 and Figure 2.

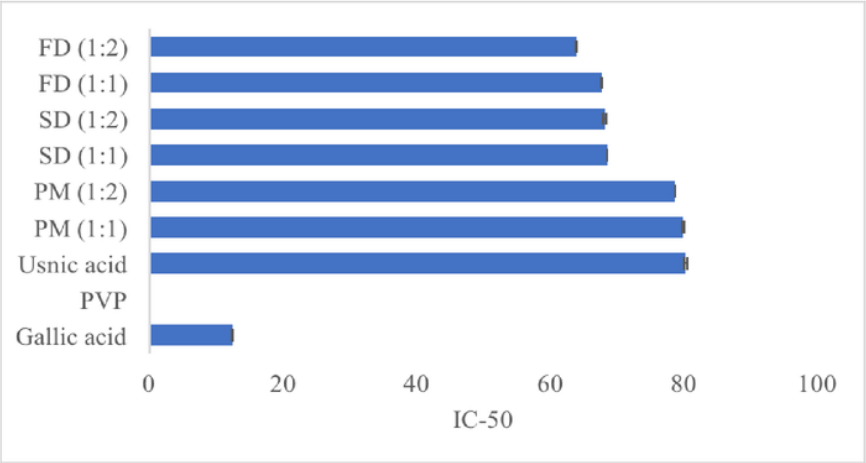

Figure 2. IC_{50} value of each sample

The data of antioxidant activity test result was statistically proceed using one-way ANOVA in SPSS 22 program. There were significant differences ($p<0.05$) in IC_{50} values of intact materials to physical mixture and solid dispersions. The enhancement of antioxidant activity is determined by concentration of usnic acid dissolved. Freeze dried usnic acid had highest the solubility and in accordance to antioxidant activity. This result was also found in freeze dried of *Uncaria Tomentosa*[11].

CONCLUSION

Solid dispersion of usnic acid – PVP K-30, which prepared by spray and freeze drying methods, is able to modify the physicochemical properties that increase the solubility of usnic acid. Freeze dried powder has the highest solubility and antioxidant activity.

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