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Roles of Hydroxyl Ions in Water-Based Solvents to Isolate Antioxidant Constituents of Natural Products

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ABSTRACT

Roles of hydroxyl ions in water-based solvents to isolate antioxidant constituents of natural products were studied in present research. Sarang semut (*Myrmecodia pendens*) tuber as the model material of the study was considered to be the strategic natural product based on its biochemical and therapeutical effects. Water with pH 3.0, 5.0, 7.0, 9.0, 11.0, and 13.0 were used as the solvents in macerations. Results showed that water with various working pH applied yielded the pH of each resulting extracts equaled to 7. The antioxidant levels of the resulted extracts indicated that higher the working pH, higher antioxidant activity of the resulted extracts. The extents of the antioxidant activities mimicked their antibacterial activities. The study also found that higher pH of the working solvent, higher amounts of the antioxidant/antibacterial extracts. **In conclusion:** the hydroxyl ions of water-based solvents applied in a maceration technology play critical role to promote the concentrations of extracts isolatable from the natural products as well as the antioxidant and/or antibacterial activity of the isolated extracts.

Keywords: analytical method, electrophilic-nucleophilic attractions, matrix-OH, matrix-H, secondary metabolites

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1. Introduction

Natural products are organic molecules that include the primary and secondary metabolites produced naturally by any organism (Cutler and Cutler, 2000; Buchanan et al., 2000). The benefits of secondary metabolites produced by the plants are to defend their selves against a variety of herbivores and pathogenic microbes. Some secondary metabolites serve other important functions as well, such as providing structural supports, as in the case of lignin, or acting as pigments, as in the case of the anthocyanins (Taiz et al., 2015), or acting as an antioxidant in the case of Vitamin E (α -tocopherol). The antioxidant activities of natural product extracts are connected with their free radical scavenging performance, metal chelating ability, and inhibition of enzymes involved in the oxidative processes (Dai and Mumper, 2010). There has been reported that sarang semut (*Myrmecodia pendens*) extracts have an activity to modulate superoxide dismutase enzyme (SOD), an endogenous antioxidant enzyme, in rats' serum (Lamondo et al., 2014). This evidence indicates the beneficiary function of the extracts as a modulator of the antioxidant enzyme, and therefore the antioxidant benefits

of the extract. The mentioned report of Situmeang et al. (2015) that *M. pendens* extracts have antibacterial activity is consistent with our data found in present study. Our data indicated that antibacterial and antioxidant activities of the isolated extracts differed among the solvent pH existing within the maceration system. There was found that higher pH of the solvent systems, higher antioxidant and antibacterial activities of the isolated extracts. Hence, higher amounts of the antioxidant extracts isolatable by a protocol, higher benefits of the isolated extracts. Yet, based on those benefits of the antioxidant extracts, the *M. pendens* plant is one of the strategic models of natural product resources to study the roles of the solvent hydroxyl ions (OH^-) in isolation of antioxidant constituents of the natural products.

Compounds doing oxidation are called the oxidant; the antioxidants play to against the power of oxidants. The α -tocopherol, quercetin, and almost secondary metabolites of the natural products are the antioxidants; some might show the activity as a prooxidant. The antioxidants working in living cells include the endogenous and exogenous groups. The SOD enzyme is one of the mitochondrial enzymes of the endogenous antioxidant pool system. It oxi-

Fig. 1. The endogenous (A) and exogenous (B) reaction stages to deactivate the free radicals

dizes the $^{\bullet}O_2^-$, the negative ion radical molecule of oxygen gas, to generate O_2 neutral and hydrogen peroxide (H_2O_2) molecules (Nozawa, 2001) in a concentration-dependent manner. The H_2O_2 neutral molecule is less reactive compared to the $^{\bullet}O_2^-$ radical, the initial radical of the pool. Once the $^{\bullet}O_2^-$ radical ion-molecule oxidized by the SOD, at the same time, it's abilities to oxidize the next hydrogen in the pathway decreases. The pattern of chemical changes follows the continual reaction shown in Fig. 1A (Nozawa, 2001). At the time found a weak of endogenous antioxidant working in living cells, the exogenous antioxidant is required to survive the cells. Deactivations of free radicals by the antioxidant extracts of natural products mimic stages of reactions reported in the continual reaction shown in Fig. 1B (Musialik and Litwinienko, 2005). The antioxidant represented by the α -tocopherol deactivates the free radicals delivered by 2,2-diphenyl-1-picrylhydrazyl (DPPH) dimers, in which the color changes, purple to yellow, indicating the α -tocopherol has antioxidant activities. Either SOD enzyme or α -tocopherol compound plays as the electrophile in their reaction pattern, respectively, takes and shares the odd electron of the free radicals. Therefore, the endogenous and exogenous antioxidants play an essential role to survive the living cells.

As human age increases, the need for exogenous antioxidants, in the form of pharmaceutical extracts, is higher accordingly. In facts, the interactions between molecules in living cells, at least involve these four interactions: the ionic and hydrogen interaction as well as the interactions through the hydrophobic and the Van der Waals (Fessenden and Fessenden, 1979; Lehninger et al., 1993). Those four types of molecular interactions develop the chemical bonding in living cells. The bonding between

antioxidants and the primary molecules of the host material in plant matrix involves those types of bondings and interactions. Separations of the antioxidant constituents from the natural resources, therefore, require considering these interactions.

Antioxidant contents of plants matrix include the minor components of their wet materials. Isolation and characterization of minute quantities of these compounds from a complex mixture remain a challenging and labor-intensive process (Anonym, 2005). This is including the challenges to provide largely isolated antioxidant extracts from the plants. Numerous authors created protocols to isolate antioxidant extracts of plants based on differentiation of the solvent polarities as well as the chemical structures of the solvents (Fu et al., 2005; Markom et al., 2007; Lau et al., 2012; Gironi and Piemonte, 2011; Swantara et al., 2012; Engida et al., 2013 and 2015;). So far we searched; there had no study to report effects of hydroxyl ($^{\bullet}OH$) ions in isolation of antioxidant of plant natural products. In the present study, we attempted to develop a protocol based on the $^{\bullet}OH$ negative ions to isolate antioxidant species of natural products. When H^+ ion content of a solution is lower than $^{\bullet}OH$ ion, the solution is alkaline. Hence, the negative ions ($^{\bullet}OH$ ions) are the nucleophile species (Fessenden and Fessenden, 1979) and have a potency to absorb poor electron species from their neighboring molecules that include the electrophile species of soaked materials. In material soaking, nucleophile elements of solvents play important roles in interaction and sorption of electrophile species from the neighboring molecules; therefore, the healthy molecules of neighbors could be affected by the nucleophile species, the hydroxyl ion species. The healthy molecules involve the antioxidant and the primary

molecule/macromolecule of matrixes of the natural products, the soaked materials. The responses of the healthy molecules of the soaked materials to the OH^- ions of the water-based solvents depend on their natural structures. Higher amounts of electron-poor (electrophile) constituents of the soaked materials, higher the effects of the OH^- ion species of the solvent, and vice versa. We suspected that introduction of OH^- negative ions (a nucleophile), a donating electron species, into a bonding between the antioxidants and the primary molecules of the host material (plants) would switch the constituent of the bonding, the bonding changed from the “*antioxidant* $\rightleftharpoons$ *primary molecules*” to “*HO* $\rightleftharpoons$ *primary molecules*” and thereafter delivered the free antioxidant molecules. The acidity and alkalinity pH of the working solvents would be paired respectively with the neutral pH of the resulting extracts (i); higher pH of the working solvent, higher antioxidant activities (ii) as well as the amounts of the antioxidant constituents (iii) of the isolated extracts. Based on those hypotheses, the present research was conducted to determine the effects of solvent OH^- ions on the levels of antioxidant activities of the isolated extracts as well as the amounts of the antioxidant extractable from the primary molecular matrix of the used plant materials.

2. Materials and Methods

2.1. Materials and equipment:

The *M. pendens* tubers were collected from Forests of West Timor Island of NTT Province of Indonesia. The chemicals consisted of DPPH* oxidative radicals (CAS 1898-66-4) provided by Sigma-Aldrich. The concentrated hydrochloric acid (HCl), distilled water (DW), absolute ethanol solvent, and sodium hydroxide (NaOH) chemicals were used in the analytical grades. Whatman 42 filter paper was used for filtration.

The equipments include: an FD-100 Eyela freeze-dryer machine was used to eliminate the water of the required sample; a Cary 100 Agilent UV-Vis spectrophotometer equipped with a personal computer was to read the spectrometrical absorbances; the ultra-performance liquid chromatography quadrupole time of flight mass spectrometric (UPLC-QTOF-MS/MS) machine was used to determine the constituents of the isolated extracts from various working pHs.

2.2. Methods:

2.2.1. Preparations of reagents, solvents, and samples for absorbance reading

2.2.1.1. Preparation of DPPH* reagent

Fixedly 1.20 mg of the DPPH* (MW 394.32 g/mol) was weighed using a digital balance with an accuracy of 10^{-6} g (0.000000 g). Then totally and accurately, it was moved to a volumetric flask 25 mL containing 10 mL of absolute ethanol. The flask was filled up with absolute ethanol until

limit sign. The flask was closed and labeled with 25 mL DPPH* 120 μM . The solution was mixed vigorously and put in a cool box containing solid ice.

2.2.1.2. Preparations of hydroxyl ions-containing solvents

To treat the pH effects in maceration of the plant materials, the present study used various pHs of DW solvents that included pH 3.0, 5.0, 7.0, 9.0, 11.0, and 13.0. The DW solvent pH 3 was prepared by following procedure. Into an erlenmeyer 100 mL was poured 90 mL of a DW solvent. Few drops of HCl 1 N were added into the erlenmeyer containing 90 mL of the DW solvent being stirred with a magnetic stirrer. The pH meter was used to read the pH of the solution being prepared. NaOH 0.1 N solution was used to adjust the required pH 3, then filled up to 100 mL. Thereafter, the erlenmeyer was labeled with 100 mL DW solvent pH 3, closed, and kept in a cool box. The same protocol was applied in the preparation of the pH 5 DW solvent. The pH 9, 11, and 13 were prepared accordingly. However, the few drops of NaOH 1 N were firstly added into the erlenmeyer containing 90 mL of the DW solvent. The HCl 0.1 N solution was used to adjust the required pH and then the mixture was filled up. The erlenmeyer was labeled with 100 mL DW solvent pH 9, 11, and 13, respectively, closed and kept in the cool box.

2.2.1.3. Preparations of *M. pendens* sample

The *M. pendens* is one of the species of the *Myrmecodia*, a genus of the *Rubiaceae* family, a parasite plant (Huxley, 1978). For the present study, the plant tubers were searched and taken from the Forests in July-August (2016), brought to the laboratory and kept at room temperature for 3–4 days. The targeted parts of the plant used in the study were their tubers. The tubers were washed with running tap water and then rinsed with DW to remove any adsorbed contaminant from their surface. The cleaned tuber was then dried and cut their leather with a cutter/knife and sliced. These tuber slices were dried under sunshine for 3 days. The dried slices were ground to become powder.

2.2.2. Determined effects of solvent pH on pH of the resulting extracts

The acidity and alkalinity of DW solvents were prepared according to the subsection 2.2.1.2. Each 0.150 g of powder was put into 12 reaction tubes 20 mL that were labelled 3-1, 3-2, 3-3, 5-1, 5-2, 5-3, 7-1, 7-2, 7-3, 9-1, 9-2, 9-3, 11-1, 11-2, 11-3, and 13-1, 13-2, 13-3, respectively (n = 3 for each pH). Thereafter, 5 mL DW solvent pH 3 was added to each tube labeled 3-1, 3-2 and 3-3; 5 mL DW solvent pH 5 was added to each tube labeled 5-1, 5-2 and 5-3. The similar procedure was performed for the pH 7, pH 9, 11, and pH 13. Each mixture was mixed vigorously. All these tubes were put into 78 °C water media and incubated for 30 min. The mixture was filtered with whatman 42 filter paper, and the filtrate was kept in another reaction tube with

a suitable label. The pHs of the filtrates were recorded.

2.2.3. Determined effects of solvent pH on antioxidant activities of the isolated extracts

The DPPH method is known as the highest sensitive and selective method among others in *in vitro* to study the antioxidant activity (Brand-Williams et al., 1995). In the present study, concentrations of the DPPH* solution was prepared to be 120 μ M (subsection 2.2.1.1); the absorbance was read at the maximum wavelength, λ_{max} , that was 482 nm. The filtrate which its solvent was not eliminated, contents of added DPPH* in a cuvette was considered to be higher than the antioxidant contained within the extracts. This was indicated by the constant purple colors existing at the final reading (absorbance constant). The antioxidant activity, reported in percentage (% antioxidant activity), was calculated according to a continual equation (Garcia et al., 2012).

% Antioxidant activity

$$= \left\{ \frac{\text{Abs DPPH}^* - \text{Abs sample}}{\text{Abs DPPH}^*} \right\} \times 100\%$$

In fact, the equation above is able to apply when the purple color still appears within the cuvette at the constant absorbance (final reading). The purple color indicates the mixture remains DPPH* species in cuvette or DPPH* content is larger than the antioxidant species, in oppositely with the yellow color indicating the antioxidant contents of the extracts is larger than the content of the added DPPH*. The antioxidant activities of the isolated extracts were determined spectrometrically with the protocol in following procedure. To reading absorbance, the sample sizes in each experiment were performed according to the cuvette sizes. Because of total volume required by the cuvette size equals to 2 mL, the sample solutions were prepared according to following procedures: 1 mL ethanol and 1 mL DPPH* 120 μ M were put in the cuvette; mixed vigorously as fast as possible, close the cover of the machine. The constant absorbance was recorded in a log book. Thereafter, the Abs sample was prepared accordingly but the 1 mL ethanol was replaced with 1 mL isolated extract. The solution without the antioxidant constituent was used as a blank.

2.2.4. Determined effects of solvent pH on qualitative and quantitative compositions of the isolated extracts

The chemical compositions of the extracts macerated at various pH that involved 5, 7, 9 and 13 were respectively determined using UPLC-QToF-MS/MS (waters) instrument at the BPPT Laboratory, Banten, Indonesia. The alkaline pH was considered to be investigated because of the alkalinity pH strongly influence the antioxidant activities of the extract while acidity pH had fewer effects. Therefore, the pH 3 was considered to be ignored from the investigations. The instrument was equipped with an Acquity UPLC BEH C18

(1.7 μ m, 2.1x50 mm) column under the following conditions: flow rate = 0.3 mL/min; injection volume = 5 μ L; temp. = 40°C; eluent (mobile phase) = A: H₂O+0.1% formic acid, B: acetonitrile + 0.1% formic acid; and time (min) at min 0-1 = 95% A + 5% B, min 6-7 = 100% B, and min 7.5-9 = 95% A + 5% B.

2.2.4. Statistical analysis

Data were expressed as mean $\pm$ standard error of the mean. Data were analyzed by one-way analysis of variance, and all differences were inspected by Duncan's new multiple-range test using SPSS statistical software. The minor (single) data was slightly reported as their original values. $P \leq 0.01$ was considered statistically significant.

Fig. 2. Some secondary metabolites containing oxygen in their molecular structures (Taiz et al., 2015)

3. Results and Discussion

3. 1. Effects of the solvent pH on pH of the resulting filtrates

The power of pH to change the acidity or alkalinity of the maceration system depends on contents of the H⁺ or OH⁻ ions in a system. Considering the sizes of these ions compared to the sizes of the smallest molecules of the soaked plant material in a solvent, their sizes are less than the sizes of the secondary metabolites, such as terpenoid, phenolic, flavonoid, steroid, saponin, tannin, and alkaloid compounds, of the soaked plant materials. Therefore, either H⁺ or OH⁻ ions could migrate everywhere throughout the system and penetrate into the matrix of the soaked materials. Looking deeply the structures of these secondary metabolite compounds (some reported in Fig. 2), their bonds linked with the surface structures of the host primary molecules include through nucleophilic-electrophilic interactions. Almost secondary metabolites contain oxygen elements in

their chemical structures. The hydroxyl, oxo, and carbonyl groups are all oxygen-containing group which is the nucleophile parts of the secondary metabolites. This is in agreement with the reports stated that the antioxidant properties of the products mostly attributed to the phenolic hydroxyl groups attached to ring structures (Dai and Mumper, 2010; Lemus-Mondaca et al., 2012). Hence, the surface active sides of the host primary molecules play as the electron poor sides (electrophiles). The H^+ ion moves far away from this active sides while the OH^- ion is attracted by this surface active sides of the matrix. So far we searching, this might be the first time and the first explanation to describe the secondary metabolites interactions within their matrixes in natural products. These OH^- ions interactions are possible to destroy the original bonds between the secondary metabolite compounds and the surface active sides of the primary molecules of the soaked material. Once these nucleophilic-electrophilic interactions break, the secondary metabolites move away from their original position.

The data found in the present study indicated that maceration conducted within pH 3 (extracted at pH 3), the pH of the resulting extract equaled to 7. In facts, all pHs of the resulting extracts equalled to 7 that involved the extraction performed at pH 3 (Extr-1), pH 5 (Extr-2), 7 (Extr-3), 9 (Extr-4), 11 (Extr-5), and 13 (Extr-6), respectively (Fig. 3). These pH changes conclusively indicated that either H^+ or OH^- ions completely interacted with the bonding between the molecular units of the soaked materials. The excess H^+ ions of the solvent (under pH 7) possibly competed with electrophile secondary metabolites (larger size than the H^+ ions) being bonded with a nucleophilic position of the primary molecule of the host matrixes. Because of H^+ size smaller, the primary molecule of host matrixes released electrophile secondary metabolites and developed a new bonding with H^+ to form matrix—H new bonds.

The mechanism discussed above was possible

because the facts indicated that maceration performed in working pH under 7 yielded the pH 7 of their resulting extracts (Extr-1 and Extr-2, Fig. 3A). Based on the electrophilic properties, the acidic solvents possibly act to isolate pro-oxidant molecules of secondary metabolites from the natural products. On another hand, the excess OH^- ions (upper pH 7) possibly competed with the nucleophile secondary metabolites (larger size than OH^- negative ions) being bonded with nucleophilic position within primary molecule of soaked host matrixes; as the results the primary molecule of the host released nucleophile secondary metabolites and formed matrix—OH new bonds. As those of acidic pH, the excess OH^- ions of the alkaline solvents in maceration system gradually decreased until pH of the resulting extracts equaled to 7. This mechanism was possible because the alkaline working pH yielded the pH 7 of the resulting extracts (Extr-4, Extr-5 and Extr-6, Fig. 3A). Hence, the alkaline solvents possibly acted to isolate antioxidant molecules of secondary metabolites of the natural products.

3. 2. Effects of various working pH of water-based solvent on antioxidant activities of the isolated extracts

The various working pH applied in maceration system and resulting a neutral single pH of their extracts respectively were already described in subsection 3.1 above. Although their resulting extracts were all at pH 7 their antioxidant activities were depended on their extractions working pH, respectively. As shown in Fig. 3B, higher the working pH of the maceration, higher the antioxidant activity of the yielded extracts, and vice versa. These data indicated that the working pHs of the maceration system played a critical role in isolation of the antioxidant constituents of the *Myrmecodia pendens*, the treated natural products in the study.

Fig. 3. The working pH (A) of maceration (color curve) and the pH of the resulting extract (black curve) and the inhibitions of DPPH* oxidative radicals by the extracts (B): at various pH levels

The *M. pendens* powder (30 mg/mL) was macerated with DW solvents at pH 3.0, 5.0, 7.0, 9.0, 11.0 and 13.0 for 30 min at 78 °C (n = 3). The pHs of resulting extracts were constant that was 7 (Fig. 3A). The highest inhibition occurred at the highest pH of the maceration (Fig. 3B). The ^{a-f} different letters indicated the mean differences were significant at $P \leq 0.01$.

Those facts found in the present study are in agreement with the reports from the previous study conducted by Hadisaputra et al. (2017) who studied the effect of electron- donating group (nucleophiles) on the corrosion inhibition performance. They reported that nucleophile exhibits the highest inhibition efficiency, whereas the electrophile exhibits the lowest inhibition efficiency. The facts were also supported by the reports of Widjajanto et al. (2017). The authors reported that incubation for 18 days of vegetables with rotten fruits $\geq$ 50% of the mixture contents could produce alkaline pH of its liquid organic generated. The higher pH of the liquid organic fertilizer was accompanied with higher N (ammonia) contents of the liquid.

Based on those reports (Hadisaputra et al., 2017; Widjajanto et al., 2017), the higher antioxidant activity of the higher working pH of the plant resource used in this study, therefore, might indicate the nucleophile antioxidants of the plant resource larger than their electrophile pro-oxidant molecules. The increasing antioxidant activities of the isolated extracts conformed with increasing the working pH of the maceration system might indicate also that it was associated with enhancements of the switching bonding from the nucleophile antioxidant to the primary molecules of the matrixes, (“nucleophile antioxidant $\rightleftharpoons$ primary molecule” to be “OH $\rightleftharpoons$ primary molecule” of the matrixes), and therefore, the matrix of the primary molecules released nucleophile antioxidants from the soaked materials. These facts described the enhancement of the antioxidant compounds migration from the host matrix to the solvent phase of the maceration system conformed with the increasing pH of the maceration system. These facts also indicated formation of the matrix—OH bonds and the numbers of the matrix—OH growth accordingly (upper pH 7). As the results, the OH^- contents of the working solvent decreased, the pH of the resulting extracts equaled to pH 7. The formation of the matrix—H was lower than the formation of the matrix—OH. Overall, the typical secondary metabolites of the *M. pendens* conclusively indicated that its nucleophiles secondary metabolites were larger than those electrophiles. Moreover, to confirm these data, the antibacterial activities of those extracts were evaluated using *E. coli* at limited numbers, the isolated extracts respectively from the working pH of 5, 7, 9, and 13. These isolated extracts were incubated with *E. coli* bacteria overnight followed the protocol reported (Nascimento et al., 2000). Their results described that higher working pH, higher antibacterial activities of the extracts (data not shown).

The role of the OH^- ions is to exchange the position of the nucleophile secondary metabolites in matrixes of soaked materials. This occurs relatively easily because these OH^- ion sizes are relatively smaller than those of the nucleophile secondary metabolites. More intensive interactions of the primary structural macromolecules with

the OH^- ions enhance the release of secondary metabolites from the host material into the fluid system of the maceration. Higher antioxidant performances of the extracts reported in Fig. 3B conclusively indicate a higher promotion of the secondary metabolite deliverance from the material soaked into the fluid system. This supports the fact that secondary metabolites have antioxidant activities. This phenomenon was supported by the fact that although solvents applied the various working pH levels during maceration, the pH of the resulting extracts equal to 7 (Fig. 3A). Overall, greater the increase of the OH^- of a maceration system, greater the increase in the amounts of secondary metabolites migrated into the fluid system and enhances the antioxidant performances of the extracts.

3. 3. Effects of working pH of water-based solvent on chemical compositions of resulting extracts

The chemical compositions of the extracts involve the quantitative contents and qualitative constituents (kinds) of the compounds existed within the extracts. The quantitative contents include the yields and the antioxidant levels of the extracts. The antioxidant activities reported in Fig. 3B include to the quantitative compositions of the extracts. Those antioxidant data indicated that higher pH of the solvent, higher inhibition of DPPH* radicals by the extracts; higher pH of the DW solvent, higher performances of the extracts as anti DPPH* oxidative radicals. The plausible reasons for these facts are that these soaked materials contain large active sites (rich cloud electrons) or nucleophiles within their secondary metabolites bonded with the primary structural macromolecules (poor electrons) of the matrixes, the primary structural macromolecules play as their electron-poor constituents (electrophiles). These electron-poor constituents might act as the electrophilic portions of the soaked materials. Likewise, higher pH of the solvent, higher its OH^- ion content, and thus, the OH^- groups act as the nucleophilic portion of the solvent. Because of OH^- ions have smaller sizes than the secondary metabolites, their succeed penetrations make disturbing interactions between secondary and primary molecules in matrixes of the soaked materials. The OH^- ions competed to secondary and therefore gradually switch the interactions forward to OH^- ions. The new electrophilic-nucleophilic attractions between OH^- ions and the electron-poor sites (surface active sides) of the matrixes formed. These new electrophilic-nucleophilic attractions could attenuate the bond strength between the secondary metabolites and primary macromolecules (“secondary metabolites $\rightleftharpoons$ primary macromolecules”) of the soaked materials.

Based on Fig. 3B, the inhibitions of the extracts of acidic solvents were smaller than those extracts of alkalinity solvents. Therefore, to elucidate the chemical compositions of the extracts, the macerations were performed at pH 5, 7, 9 and 13 that were similar to those several pHs applied in Fig.

3. The standard was used quercetin which was to get the qualitative and quantitative compositions of each peak established. The quercetin standard consisted of three derivative compounds (Fig. 4), purity more than 95%, that was quercetin-3-O-rhamnoside, 3,3',4',5,6-Pentahydroxyflavone, and 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one. The effects of pH on chemical compositions were shown in Fig. 5 that involved the areas of each peak reported by the total ion chromatography (TIC) charts of the Liquid Chromatography. The typical chromatograms reported in Fig. 5 (pH 5, 7, 9, and 13) indicated that these charts look no differences in their retention time due to the various working pH. The sharp peaks among these four solvents (pH = 5, 7, 9 and 13 solvents) almost appeared at similar retention times, which were 0.25 to 0.75s namely Group I, 3.50 to 5.25s (Group II), 6.0 to 7.25s (Group III), and 7.75 to 8.0s groups (Group IV). The major compounds isolated from the materials were the compounds appearing in Group II, followed by those of Group IV.

Fig. 4. The quercetin and its derivative molecular structures constructing standard quercetin of the study bought from Sigma

Fig. 5. The chemical compositions of the resulting extracts from various working pH

The *M. pendens* powder (30 mg/mL) was macerated with DW solvents at pH 5, 7, 9, and 13 for 30 min at 100 °C. Their major peaks almost appeared at the similar retention times. The peaks with a retention time of 3.74 min detected within the extracts were equaled with that of the quercetin standard indicating a similar compound.

The retention times (tR) of the peaks detected from the extracts mimicked the retention times of the quercetin standard. Based on the peaks shown within the TIC, those three quercetin derivative compounds appeared on the TIC chart. The 3.74 retention time was one of the major peaks between the detected peaks of the extracts and the standard. These data indicated that the method used could isolate almost all of the quercetin and its derivative compounds from the bulk material of the *M. pendens* powder. The isolated extracts generated by the method might provide the antidiabetic extracts from the *M. pendens* plants, there was because the previous researchers (Arias et al., 2014) reported that quercetin compounds have the capability to reduce insulin resistance.

The variation of the pH did not influence the qualitative chemical compositions migrating from the soaked materials into the fluid system, but increasing the working pH could enhance the quantitative chemical compositions of the extracts (Fig. 5). The peaks at tR of 3.74 and 3.86 qualitatively constant at those applied pHs. At pH 5, the peak areas of tR 3.74 equal to 188 while the tR 3.86 equals to 368. Then, the pH solvent was increased to pH 7, data result showed that peak areas of tR 3.74 and tR 3.86 were 254 and 381, respectively. The pH solvent equalled to 9, the peak areas of tR 3.74 was constant equalled to the solvent pH 7, but the tR 3.86 equalled to 461. Higher again the solvent pH to 13, the peak areas of tR 3.74 and tR 3.86 were 505 (5x101) and 1,725 (5x345), respectively. These results indicated that pH of the water-based solvent is one of the critical determinants affecting contents of antioxidant as well as prooxidant extractable from the plant materials. In opposite study reported that acidic pH promotes the synthesis of phenolic compounds in various tissues (Keller et al., 1997; Radić et al., 2016), while our study reported that alkaline pH plays essential role to isolate antioxidant extracts, including the polyphenols, of natural products from the used plant in the study (Fig.3 to Fig. 6).

4. Conclusion

The H⁺ ions of solvent play as the electrophilic constituents and therefore competed with electrophilic parts of the targeted soaking materials, while the OH⁻ ions play as the nucleophilic constituents and competed with their nucleophilic parts. The H⁺ ions contribute to isolating the electrophile and therefore the isolated compounds are the pro-oxidants, while those interacted with the OH⁻ ions are antioxidants (nucleophile) and therefore are the antioxidants. The data found in the present study indicated that larger pH of the solvent applied, larger the scavenging activities of the extracts isolated from the bulk materials of the products. Based on these facts, therefore, the hydroxyl ions of the water-based solvents play critical roles to isolate antioxidants constituents of the natural products.

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