A Novel Application of Solubility Parameter in Extraction of Bioactive Substances from Natural Products

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Abstract

The method to design the solvent for the optimal extraction of bio-active ingredients from natural resources was developed. The extraction of polyphenols, anti-oxidant and anti-tyrosinase from mulberry leave was varied with the solvent species and composition of the binary mixture. Due to their own polarities of the active ingredients, the optimal solvent composition of alcohol-water mixture was achieved between 40 – 80 % alcohol fraction depending on the alcohol species and target active ingredients. As such, for the extraction of the polyphenols and anti-oxidant the optimal alcohol fraction of alcohol-water binary solvent was about 60 % methanol and was lower to about 40 % with ethanol and propanols due to their low polar propensities. For the extraction of the anti-tyrosinase ingredients, a higher alcohol fraction of the optimal solvent condition was required because of the lower polarity of target ingredients than that of the anti-oxidants.

The extraction efficiency of the bio-active ingredients was correlated with solvent polarity represented with solubility parameter. According to correlations, the optimal solvent polarities for the extraction of polyphenols and anti-oxidation and anyi-tyrosinase ingredients were predicted as 41.2, 40.3 and 35.1 [$MPa^{1/2}$], respectively. These solvent polarities were well agreed with the optimal solvent conditions of alcohol species and composition on the binary mixture. Also, the correlation was confirmed with model solvents design with other solvent species of acetone and ethylene glycol.

Introduction

Recent studies on exploiting the natural ingredient for medicals and cosmetics have drawn much attention to the effective extraction of desired bio-active ingredients from natural resources acting on the human metabolism. Typically, various solvents of water, alcohols, acetone and ether etc. were used to extract out bio-active substances included in the natural resource because they have polarities in broad range. As such, the water was applied for extraction of high polar ingredients of carbohydrate, glycoside and amino acid, and the ether for low polar ones of aromatic compounds. Also, the solvent of alcohol-water mixture was also adopted to adjust the polarity of extraction solvent. Then, it would be followed to investigate a molecular compound of specific functionality from the extraction.

For examples, Doi *et al*. [1] have used hexane and butanol from root of mulberry and then to investigate the specific bio-active ingredients of prenyflavas, glycoside, iso-quercetine and astragalin, which might be favored to low polar solvent. Methanol is frequently used to investigate some bio-active ingredients from various natural resources. As such, the anti-inflammatory ingredients are found from methanol extraction of *Culcasia scadens* P. Beauv [2], the anti-microbial compounds from *Ceanothus americanus* [3] and the anti-histaminic compounds from *Mentha spicata* [4]. Also, the ethylacetate and n-hexane are applied to extract bio-active ingredients. From above previous studies of investigation it is implied that the solvent polarity must be important to extract just specific functional ingredient out of the natural resource. Thus, various solvents, pure or mixture, were applied to extract the bio-active ingredients having various polarities [5].

The study to search the optimal extraction condition for the bio-active ingredients of anti-oxidation from *Spirulina platenis* was performed [6]. That is, ethanol was suggested as the best solvent among hexane, petroleum ether and water and the extraction temperature and time influenced barely on the extraction of the anti-oxidation. According to Liyana-Pathirana *et al*. [7], using methanol, ethanol and acetone, it was attempted to find an optimal solvent condition for extraction of polyphenolic compounds from wheat by varying with solvent concentration and extraction temperature and time. From the previous studies, although it was shown that the optimal extraction condition was found by various trials with various solvents of species and compositions, it was not enough to develop a general method to determine the solvent condition for the optimal extraction, which could be applied to natural resources.

Using mulberry leave in the present study, it will be attempted to develop a method to determine the optimal solvent condition for the extraction of bio-active ingredients from natural resources and to design the solvent for the optimal extraction. Since the extraction of specific ingredient from the natural resources depended on the polarity of solvent, as implied in the previous studies, the extraction efficiency of the solvent to bio-active ingredients is investigated along with solvent condition of polarity varying with species and composition of binary alcohol-water solvent. Here, methanol, ethanol, n-propanol and iso-propanol are used as alcoholic species for the binary mixture. As already known that mulberry leave contains effective ingredients specific to anti-oxidation and anti-hyperpigmentation, they will be references to evaluate the extraction efficiency of solvent [8, 9].

Experimental Procedure

Extraction

Mulberry (*Morus alba* L.) leaves, which was purchased at herbal market in Korea, were completely dried using oven at 60-80°C for couple of days and then finely pulverized with a milling machine. The powder of the leave was meshed with aperture size of 200 μm and kept in desiccators. To obtain the bio-active ingredients the leave of 2 g was extracted using 10 ml of solvent made of alcohol and water binary mixture for 1 hr in hot bath of 80 °C. Then, extracted solution separated from solid leave using centrifuge (Hanil science industrial Co., Ltd. HA-500, Korea) was analyzed the contents of bio-active ingredients such as polyphenolic compounds, anti-oxidant and tyrosinase inhibitor.

The solvent condition to extract the bio-active ingredients was varied by adjusting the alcohol composition and species in the alcohol-water binary mixture. Methanol, ethanol, n-propanol and iso-propanol were used for the binary mixture and their compositions were changed from 0 to 100 %. Further, dimethylformamide, and acetone were also applied to formulate the binary mixture solvent to evaluate the optimal solvent condition for the extraction. For the present experiment, all chemicals except some were purchased from Sigma-Aldrich Chemical Co (U.S.A.) with ACS grade.

Assay of phenolic compounds

Based on Goldstein and Swain method [10], the total content of polyphenolic compounds in extraction was evaluated. First, the extraction was diluted fifty times with distilled water (1/50 (v/v)) and then sample of 100μl was completely mixed with 1,250 μl of the Folin-Denis reagent (ACS grade, Fluka, Switzerland), which was diluted ten times with distilled water. Then, the mixture solution was incubated at 25°C for 20min after adding the saturated sodium carbonate of 250 μl. Using UV spectrophotometer (JASCO, Model V-570, Japan) the absorbance of 760 nm wavelength was measured to estimate the content of polyphenolic compounds by standard concentration curve of tannic acid which exerted equivalent absorbance of the same wavelength. The standardization of the tannic acid was prepared with the same procedure for measurement of UV absorbance of the sample extraction. The tannic acid solution of 100 □l was mixed with the same Folin-Denis reagent of 1,250μl and incubated with the same conditions. The measurement of the UV absorbance of the tannic acid solution resulted in the standard curve as follows,

$$C_P = \frac{(A_T - 0.0198)}{0.00472} \qquad (1)$$

where C_P was the tannic acid concentration [g/ml] and A_T was the UV absorbance. Using eq. (1), the polyphenol concentration equivalent to the tannic acid one was inferred and then the content of polyphenols extracted from unit mass of mulberry leave was estimated.

Anti-oxidation activity

The anti-oxidation activity of ingredients extracted was evaluated by degree of scavenging 1,1-diphenyl-2-picryhydrazyl (DPPH) free radicals, as suggested by Xiong *et al*. [11]. First, free radical solution was prepared with 0.15 mM DPPH in ethanol of 2,000 μl and 0.5%(v/v) Tween-20 solution of 100 μl. Then, pH of the radical solution was adjusted to 7.4 using 0.1M Tris-HCl buffer of 1,800μl. After adding the sample extraction of 10μl to the radical solution, it was allowed to react for 30 min at room temperature before measuring the UV absorbance at 517 nm wavelength. As the reference of UV-absorbance to the base of anti-oxidation activity, the blank solvent, which did not contain an extract, was used. Then, the activity of the extraction (C_{AO}) could be expressed in relative scale as,

$$C_{AO} = \frac{A_{AO} - A_{RO}}{A_{RO}} \times 100(\%) \qquad (2)$$

where A_{AO} and A_{RO} were UV absorbances of sample extraction and blank solvent, respectively.

Anti-tyrosinae acitivity

Based on a method suggested by Lee *et al*. [9], the mushroom tyrosinase inhibition of the ingredients extracted was measured as indicative activity of anti-hyperpigmentation. The sample extraction was diluted in 50 % (v/v) with methanol. Then the diluted sample of 320 μl was mixed with 0.83 mM *L*-dopa of 960 μl and the tyrosinase solution of 320 μl, which contained the 125 unit/ml and buffered with 0.1 M phosphate as pH 6.8. The mixture solution was quickly cooled at 0 C right after its incubation at 37°C for 10 min and then UV absorbance of the solution at 490 nm was measured. As the reference of UV absorbance to base of anti-tyrosinase activity, the blank mixture, which did not contain a tyrosinase, was used. Then, the activity of the extraction (C_{AT}) could be expressed in relative scale as,

$$C_{AT} = \frac{A_{AT} - A_{RT}}{A_{RT}} \times 100(\%) \quad (3)$$

where A_{AT} and A_{RT} were UV absorbencies of sample extraction and blank mixture, respectively.

Results and Discussion

1. Extraction of bio-active ingredients

It is known that most bio-active ingredients of natural plants belonging to the polyphenolic compounds, such as mulberroside F, quercetine etc in mulberry leave, have broad solubility propensities on solvents. In the present study, thereby, the various alcoholic solvents with various compositions were applied to achieve the most effective extraction condition for bio-active ingredients of mulberry leaves. The extraction efficiency of the solvents was evaluated in three terms of total polyphenolic content, anti-oxidation acitivity (DPPH radical scavenging) and anti-hyperpigmentation activity (mushroom tyrosinase inhibition) of the extracted solution.

As shown in Fig. 1, the total content of polyphenols extracted from the mulberry leave varied with the composition and the species of alcohol in the binary mixture solvent. The extraction efficiencies of propanol binary mixtures (n-propanol-water and iso-propanol-water mixtures) were slightly maximized at about 20 % of alcohol composition and then dramatically reduced with increasing the composition of propanols. Using ethanol-water mixture, the optimum condition of the extraction appeared at about 40 % of alcohol composition although the extraction efficiency was somewhat fluctuated along the alcohol composition. It was shifted to 60 % of alcohol composition in case of methanol-water mixture. Also, the dependency of the extraction efficiency on the methanol composition was much diminished in comparing with that in propanol-water mixture. It was interesting to note that the total content of extracted polyphenols varied with alcohol species and composition, it at the optimal solvent condition of each alcohol species appeared almost same.

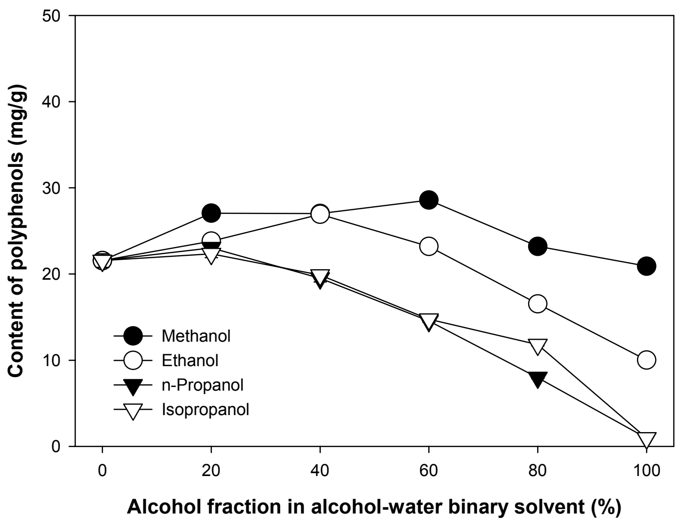


Fig. 1. Extraction of polyphenol content by alcohol-water binary mixture

Table 1. Cohesive energies and solubility parameter of solvent

solvent	$M[g\ mol^{-1}]$	$v[cm^3\ mol^{-1}]$	$\delta[MPa^{1/2}]$			
			δ_d	δ_p	δ_h	δ
Water	18.02	18.1	12.2	22.8	40.4	48.0
Methanol	32.0	40.7	11.6	13.0	24.0	29.7
Ethanol	46.1	58.7	12.6	11.2	20.0	26.1
n-Propanol	60.1	75.2	14.1	10.5	17.7	24.9
Ethylene glycol	62.1	55.9	10.1	15.1	29.8	34.9
Acetone	58.1	74.0	13.0	9.8	11.0	19.7

The extraction of organic ingredients from the plant leave was directly related with compatibility of the ingredients to the solvent; as such, the ingredients well matched in polarity with the solvent, would highly be extracted and otherwise, hardly extracted. Therefore, it would be inferred from the present experimental results that polyphenolic compounds in the mulberry leave was optimally compatible with alcohol-water mixture at a certain polarity between ones of alcohol and water, of which the composition varied with alcohol species. That is, the optimal alcohol-water mixture appeared at low composition with propanols and was shifted to high one with methanol, because the methanol was more polar than the propanols, as displayed in Table 1. Also, since the span of polarity of methanol-water mixture was much smaller than one of propanol-water mixture, it was exhibited that extraction efficiency of methanol-water mixture was much less sensitive to the composition than that of propanol-water mixture.

The anti-oxidation activity, equivalently capability of scavenging the DPPH free radicals, of extracted solution at various conditions was evaluated, as shown in Fig. 2. In case of using the methanol for binary mixture, the maximum anti-oxidation activity of the extracted solution was found at around 60 % of alcohol composition and then shifted to lower alcohol fraction as using ethanol and propanols. It was interesting to note that the anti-oxidation activity of the extracted solution was more sensitive to the solvent conditions (species and composition of alcohol) than the extraction efficiency of polyphenols. Using methanol and ethanol for binary water mixtures, especially, the anti-oxidation activity of the solution extracted above high alcohol composition (above 80 %) was dramatically reduced albeit the extraction efficiency to polyphenolic compounds remained with slight drop from the maximum extraction efficiency. From this result it could be suggested that among the polyphenolic compounds in the extracted solution the effective ingredients on the anti-oxidation were quite polar and depended sensitively on the solvent polarity.

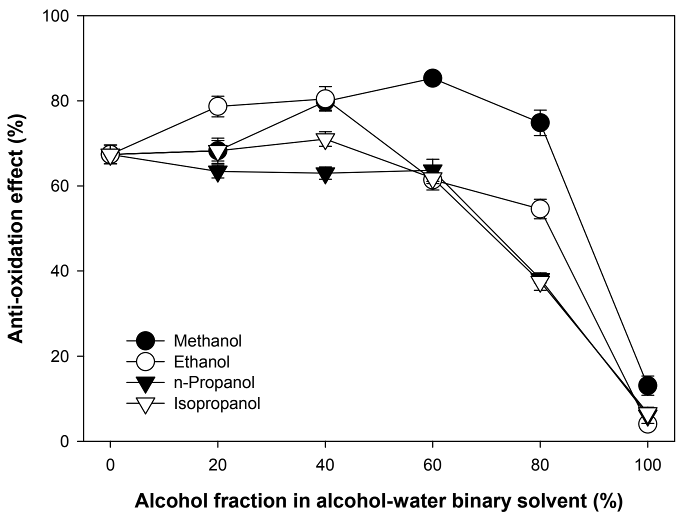


Fig. 2. Extraction of bio-active ingredients for anti-oxidation by alcohol-water binary mixture

However, the anti-tyrosinase activity of the extracted solution was differently behaved along with the solvent conditions of alcohol species and composition. As shown in Fig. 3, as increasing the methanol fraction in the solvent, the activity of the solution was monotonically enhanced up to 80 % of alcohol composition and then dramatically dropped to almost zero whereas the optimal solvent condition was shifted to below 60 % of alcohol composition in cases of ethanol, n-propanol and iso-propanol. That is, the bio-active ingredient for the anti-tyrosinase was favorably extracted by the solvent of lower polarity than polyphenolic compounds and anti-oxidation ingredients.

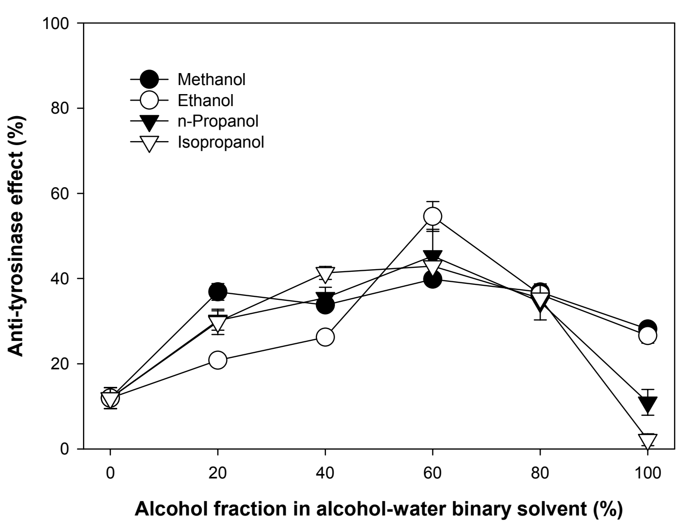


Fig. 3. Extraction of bio-active ingredients for anti-tyrosinase by alcohol-water binary mixture

2. Design of solvents for extraction of bio-active ingredients

As shown in above experiment, the extraction efficiency of the binary solvent to the bio-active ingredients was directly related with the polar propensity of solvent, which varied with the alcohol fraction and species in the mixture. That is, the optimal solvent condition to extract the specific ingredients might be expected by the polarity of the solvent mostly compatible with the ingredients. Thereby, the solubility parameter suggested by Hildebrand [12], representing simply a polar propensity of matter, was introduced to determined the extraction efficiencies of solvent to polyphenol content, anti-oxidation ingredients and anti-tyrosinase ingredients with polarity of the solvent. Actually, this parameter was frequently used to indicate the miscibility between materials [12] and to predict the solubility of matter to a solvent [13, 14] because one matter was miscible and highly soluble to the other having similar polarity with that of the matter. According to Hildebrand [12], the solubility parameter was related with the cohesive energy ($E_{coh,i}$) made of a linear combination of contributions from dispersion interaction ($E_{d,i}$), polar interaction ($E_{p,i}$) and hydrogen bonding interaction ($E_{h,i}$) was expressed as,

$$\delta_i = \sqrt{\frac{E_{coh,i}}{V_i}} = \sqrt{\frac{E_{d,i} + E_{p,i} + E_{h,i}}{V_i}} \tag{4}$$

Since the cohesion parameters were related with corresponding interaction energies as,

$$E_{d,i} + E_{p,i} + E_{h,i} = \delta_{d,i}{}^2 + \delta_{p,i}{}^2 + \delta_{h,i}{}^2 \tag{5}$$

then, the solubility parameter is derived as,

$$\delta_i = \sqrt{\delta_{d,i}{}^2 + \delta_{p,i}{}^2 + \delta_{h,i}{}^2} \tag{6}$$

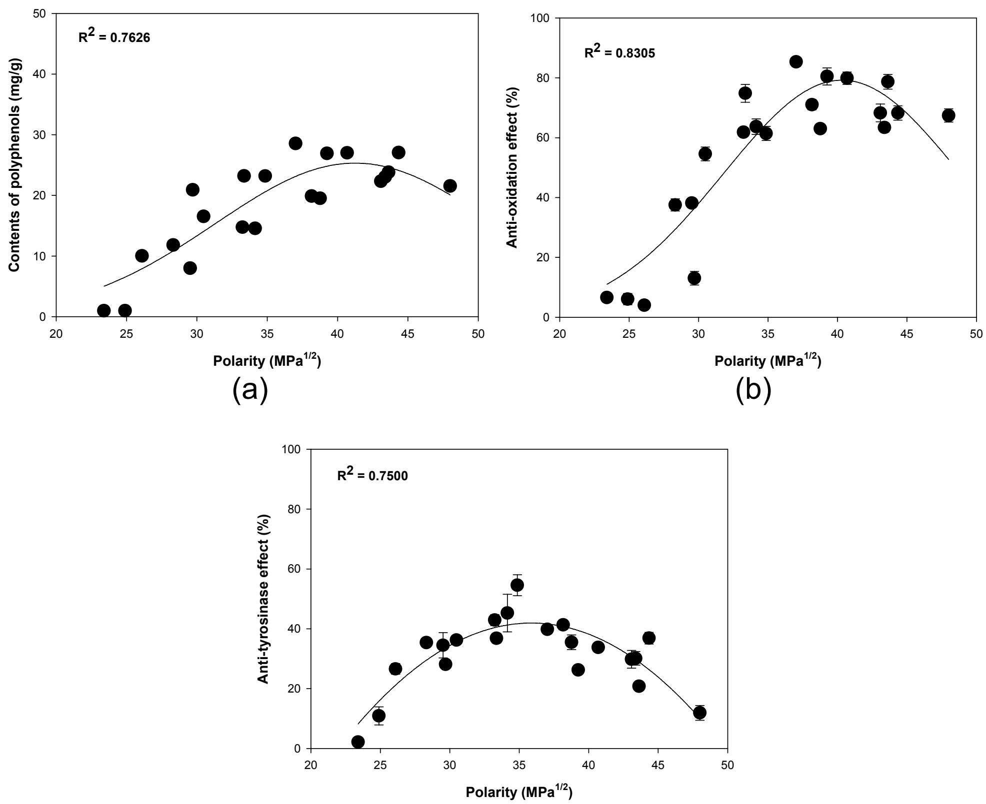
where δ_i is solubility parameter [$MPa^{1/2}$] of species *i* and V_i is mole volume of pure species. Using the cohesion parameter values, as summarized in Table 1, the solubility parameter of pure species of water and alcohols could be calculated. Then the solubility parameter of the alcohol-water mixture was estimated by simple rule of mixing as,

$$\delta_m = \sum_i x_i \cdot \delta_i \tag{7}$$

δ_m is solubility parameter of alcohol-water mixture and x_i is volume fraction of species in the mixture. In the present study, the solubility parameter value of the solvent was simply adopted as its polarity.

As shown in Fig. 4, the extraction efficiencies of solvents to the bio-active ingredients of mulberry leave, which varied with alcohol species and composition, were well correlated with single parameter of the solvent polarity. The extraction efficiency to the polyphenolic compounds were achieved the best at 41.2 [MPa$^{1/2}$] of the solvent polarity (see Fig. 4 (a)) corresponding to 36.9 % methanol fraction, 30.9 % ethanol fraction, 29.3 % n-propanol fractions and 27.5 % iso-propanol fraction of the binary mixtures. For the optimal extraction of anti-oxidation the solvent polarity was 40.3 [MPa$^{1/2}$] (see Fig. 4 (b)) resulting in slight increase of the alcohol fraction of the binary mixture (42 % methanol, 35.1 % ethanol, 33.3 % n-propanol and 31.2 % iso-propanol fractions of the binary mixtures). These experiment results of optimal solvent polarity for the extraction would explain the reason why the optimal alcohol composition was varied with the alcohol species in Fig. 1 and 2, and were also good in agreement with profiles of extraction efficiencies along with alcohol compositions.

As expected in Fig. 4(c), however, it was found that the optimal polarity of the solvent for the anti-tyrosinase ingredients was about 35.1 [MPa$^{1/2}$] lower than that for the polyphenols and anti-oxidation ingredients. Due to such low polar condition of the optimum solvent, the high alcohol fraction was necessarily required such as 70.3 % methanol, 58.7 % ethanol, 55.7 % n-propanol and 52.3 % iso-propanol fractions of the binary mixtures.



(c)

Fig. 4. Correlations of extraction of bio-active ingredients form mulberry leave with polarity of solvent. (a) content of polyphenols, (b) anti-oxidation ingredients and (c) anti-tyrosinase ingredients.

Table 2. Solubility parameters of model solvents

solvent	δ[MPa$^{1/2}$]	Symbols (for fig.5)		
		Content of polyphenols	Anti-oxidant effect	Anti-tyrosinase Effect
25% Ethylene glycol in water	44.73	●	○	⊕
42% Ethylene glycol in water	42.50	■	□	⊞
58% Ethylene glycol in water	40.40	▲	△	▲+
47% acetone in water	34.70	▼	▽	▼+
57% acetone in water	31.87	◆	◇	◆+
27% acetone in methanol	27.00	⬣	⬡	⬣+

The correlations of extraction efficiency of bio-active ingredients with solvent polarity were evaluated using the model solvents having a broad range of polarity. Using ethylene glycol, acetone, methanol and water, as summarized in Table 2, the model solvents designed for the polarity from 27.0 to 44.73 [MPa$^{1/2}$] were applied to extract the bio-active ingredients, as shown in Fig. 5. Despite the model solvents of different species and composition, if same in polarity, they exhibited the same their extraction efficiencies for the bio-active ingredients, such as polyphenol content, anti-oxidation and anti-tyrosinase ingredients and were also in good match with the above correlations obtained using the alcohol-water binary mixtures.

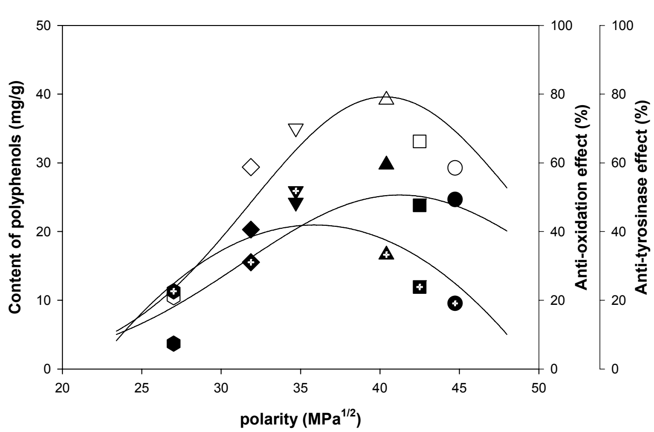


Fig. 5. Comparison of extraction of bio-active ingredients by model solvents with correlation of extraction along with solvent polarity.

Table 3. The molar vaporation energy and molar volume of functional groups for prediction of solubility parameters of quercetine and mulberroside F. [9]

Group, z	^{g}U/KJ mol^{-1}	^{g}V/cm^{3} mol^{-1}
$-CH_3$	4.71	33.5
$-CH_2-$	4.94	16.1
>CH−	3.43	-1.0
−CH=	4.31	13.5
>C=	4.31	-5.5
Ring closure, 5 or more atoms	1.05	16
Conjugation in ring, for each double bond	1.67	-2.2
−OH (disubstituted or on adjacent C atoms)	21.9	13.0
−OH	29.8	10.0
−O−	3.35	3.8
−CO−	17.4	10.8

Additionally, the polarity of quercetine and melberroside F were estimated to confirm the above correlation by method based on functional group contribution theory [12, 15]. Those compounds, of which molecular structures were shown in Fig. 6, were the most active compounds of anti-oxidation and anti-tyrosinase in mulberry leave, respectively. Without any cohesive energy data, the solubility parameter of quercetine and melberroside F could be predicted from molar vaporization energy ($^{g}U_i$) and molar volume ($^{g}V_i$) of functional group of the compound using the following equation,

$$\delta_i = \left[\frac{\sum {}^{g}U_i}{\sum {}^{g}V_i}\right]^{1/2} \quad (8)$$

Using the functional group data of molar vaporization energy and molar volume, summarized in Table 3, eq. (8) resulted in the polarity of quercetine and melberroside F as 39. 23 and 37.71 [MPa$^{1/2}$], respectively. Those values of the polarity of compound, which were consistently agreed with optimal solvent polarities for the extractions, implied reason why the optimal solvent condition for the anti-tyrosinase extraction occurred at lower solvent polarity than that for the anti-oxidation extraction in the correlations.

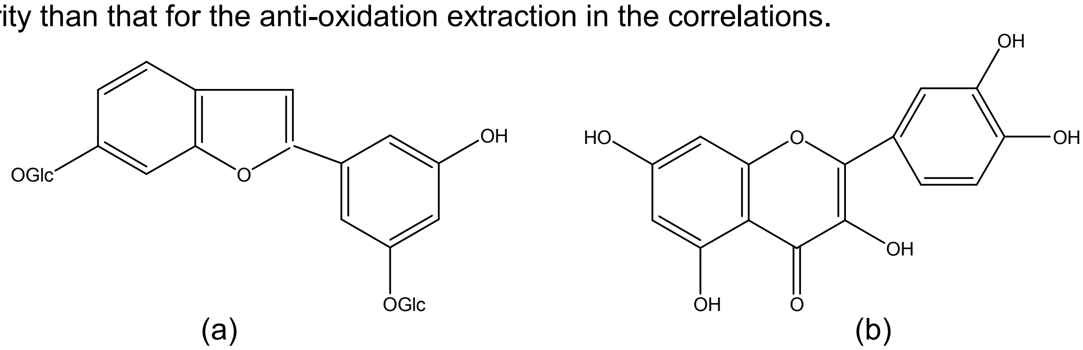


Fig. 6. Molecular structures of quercetine and mulberroside F that are most active ingredients for anti-oxidation and anti-tyrosinase, respectively, in mulberry leave.

The compatibility of the correlation with extraction of content of a specific functional compound was examined by using TLC (thin film chromatography), as shown in Fig. 7. From solutions extracted at three different solvent polarities (23.5, 34.1 and 47.1 [Mpa$^{1/2}$]), as marked on Fig. 4(b), it was clearly found that the content of mulberroside F was more highly obtained with the solvent condition of 34.1 [Mpa$^{1/2}$], which was very closed to the optimal polarity in the correlation, than with any others, although the different band distributions appeared due to different ingredients and contents of the solutions.

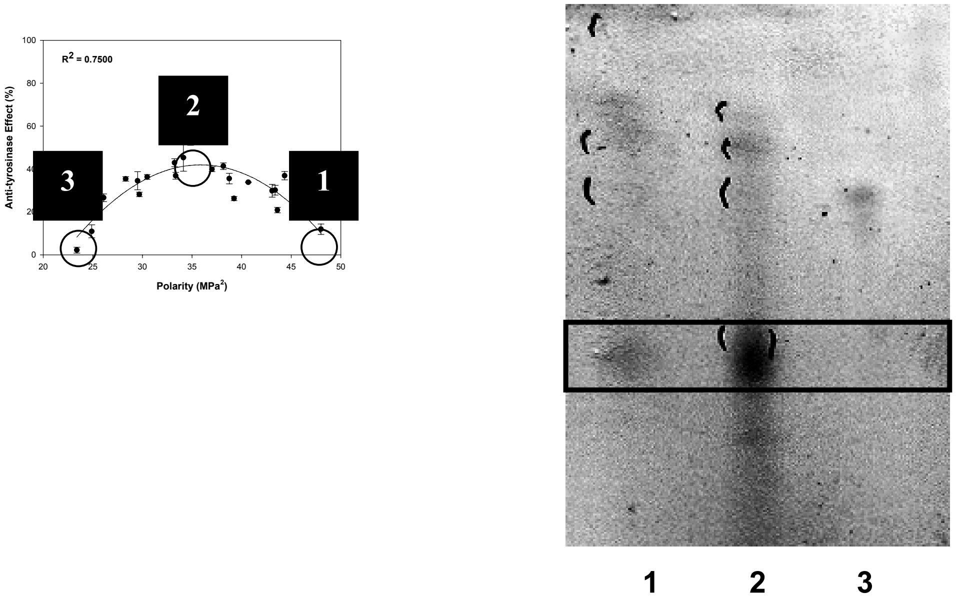


Fig. 7. Comparison of concentration of mulberroside F extacted at three different solvent conditions.

Conclusion

The effective solvent to extract bio-active ingredients such as anti-oxidant and anti-tyrosinase in mulberry leave was searched by varying with solvent species and compositions. For the effective extraction of target ingredients having specific activity among many ingredients, the solvent condition was determined with polar propensity of the target ingredients. As such, the active ingredients to the anti-oxidation and polyphenols having high polar propensities required about alcohol-water binary solvent of 60 % methanol fraction for the optimal extraction, whereas anti-tyrosinase ingredients were most favorably drawn out by the binary solvent of 80 % methanol fraction. In addition, due to the lower polar propensity of ethanol and propanols than methanol, their fraction of the binary mixture for the optimal extraction of active ingredients was lower than methanol fraction.

Such dependency of the optimal extraction on the solvent species and composition could simply be described with the solvent polarity, which was represented with solubility parameter of solvent in the present study. From those correlations of the extraction efficiency to anti-oxidation and anti-tyrosinase ingredients along with the solvent polarity, the optimal conditions of solvent were predicted as above 40.0 and 35.0 [Mpa$^{1/2}$] of solubility parameter, respectively. Those predictions of the solvent condition for extractions of anti-oxidation and anti-tyrosinase ingredients were well consistent with the model solvent conditions designed with acetone and ethylene glycol, and also confirmed by extracted contents of quercetine and mulberroside F, which were the most active compounds to anti-oxidation and anti-tyrosinase in the mulberry leave, respectively.

The correlations between extracted bio-active ingredient contents and the solvent polarity could allow to easily inducing information on the solvent for optimal extraction of target ingredients of specific activity, which might be useful for design of process as well as solvent in industry.

Acknowledgements

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