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FTIR- and UHPLC-Q-Orbitrap HRMS-based Metabolomics of *Sonchus arvensis* Extracts and Evaluation of Their Free Radical Scavenging Activity

(Metabolomik Berasaskan FTIR- dan UHPLC-Q-Orbitrap HRMS bagi Ekstrak *Sonchus arvensis* dan Penilaian Aktiviti Penghapusan Radikal Bebasnya)

MOHAMAD RAFI[1,2,3,*], TRIYANI HASANAH[4], ALFI HUDATUL KAROMAH[1], ADE HERI MULYATI[4], TRIVADILA[1,2], MIN RAHMINIWATI[5], SUMINAR SETIATI ACHMADI[1,2] & DYAH ISWANTINI[1,2]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Jalan Tanjung Kampus IPB Dramaga, Bogor 16880, Indonesia*
[2]*Tropical Biopharmaca Research Center, Institute of Research and Community Services, IPB University, Jalan Taman Kencana No. 3 Kampus IPB Taman Kencana, Bogor 16128, Indonesia*
[3]*Advance Research Laboratory, Institute of Research and Community Services, IPB University, Jalan Palem Kampus IPB Dramaga, Bogor 16680, Indonesia*
[4]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor 16143, Indonesia*
[5]*Division of Pharmacology, Department of Anatomy, Physiology, and Pharmacology, School of Veterinary Medicine Biomedical Sciences, IPB University, Kampus IPB Dramaga, Bogor, Indonesia*

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ABSTRACT

Sonchus arvensis, known as sowthistle, belongs to Asteraceae and contains numerous phenolic acids and flavonoids that exhibit antioxidants. The type of extraction solvent will affect biological activity level, resulting in different metabolite profiles that metabolomics approaches can evaluate by fingerprint analysis using FTIR spectrophotometry and UHPLC-Q-Orbitrap HRMS. Furthermore, the FTIR spectra and the metabolite profile of UHPLC-Q-Orbitrap HRMS were grouped using principal component analysis (PCA). This study aimed to clustering *S. arvensis* extracts based on different extracting solvents using the FTIR spectra and the detected metabolites and evaluate the free radical scavenging activity. The 50% ethanol extract gave higher free radical scavenging activity and more metabolites than the ethanol p.a. and water extracts. It indicates that the free radical scavenging activity is affected by the metabolites contained in the extract. Using a combination of the FTIR spectra and the peak area of the identified metabolites, *S. arvensis* extract can be grouped according to the extracting solvent. Thus, the extracting solvent affects the composition of the metabolites, resulting in different free radical scavenging activity levels.

Keywords: Antioxidant; FTIR; LC-MS/MS; metabolite profiling; *Sonchus arvensis*

ABSTRAK

Sonchus arvensis dikenali sebagai *sowthistle*, tergolong dalam Asteraceae dan mengandungi banyak asid fenol dan flavonoid yang mempamerkan antioksidan. Jenis pelarut pengekstrakan akan mempengaruhi tahap aktiviti biologi, menghasilkan profil metabolit berbeza yang pendekatan metabolomik boleh dinilai dengan analisis cap jari menggunakan spektrofotometri FTIR dan UHPLC-Q-Orbitrap HRMS. Tambahan pula, keputusan spektrum FTIR dan profil metabolit UHPLC-Q-Orbitrap HRMS telah dikumpulkan menggunakan analisis komponen utama (PCA). Kajian ini bertujuan untuk mengelaskan ekstrak *S. arvensis* berdasarkan pelarut pengekstrakan yang berbeza menggunakan spektrum FTIR dan metabolit yang dikesan dan menilai aktiviti perencatan radikal bebas. Keputusan yang diperoleh menunjukkan bahawa ekstrak etanol 50% mempunyai aktiviti perencatan radikal bebas yang lebih tinggi dan bilangan metabolit yang lebih tinggi daripada ekstrak etanol p.a. dan ekstrak air. Ini menunjukkan bahawa aktiviti perencatan radikal bebas dipengaruhi oleh metabolit yang terkandung dalam ekstrak. Menggunakan gabungan spektrum FTIR dan kawasan puncak metabolit yang dikenal pasti, *S. arvensis* boleh dikumpulkan mengikut pelarut pengekstrakan. Berdasarkan keputusan yang diperoleh, pelarut pengekstrakan mempengaruhi komposisi metabolit, mengakibatkan tahap aktiviti perencatan radikal bebas yang berbeza.

Kata kunci: Antioksidan; FTIR; LC-MS/MS; profil metabolit; *Sonchus arvensis*

Introduction

Indonesia is rich in plant biodiversity, which is an advantage for the community to prevent or treat a disease. One of them is *Sonchus arvensis*, known as show thistle or *tempuyung* in Indonesia, which belongs to the Asteraceae family. Previous studies reported that this particular species has several biological activities such as antioxidant (Khan 2012), antibacterial (Kanaani & Sani 2015), antihyperuricemic (Widyarini et al. 2015), anti-inflammatory (Prasad et al. 2015), antihypertensive (Sukandar et al. 2019), and antidiabetic (Dutta et al. 2020). Besides being used as herbal medicine, it is also consumed as fresh vegetables and functional drinks.

The various biological activities from *S. arvensis* come from several bioactive compounds from the phenolic acids, flavonoids, sesquiterpene lactones, quinic acid esters, and triterpene (Li & Yang 2018). This biological activity, such as antioxidant activity, will depend on the composition and concentration of the bioactive compounds, particularly those from the phenolic acid and flavonoid groups, most of which are widely known to have antioxidant activity (Karamian et al. 2016).

Several factors affect the composition and concentration of compounds in plants, such as extracting solvents with different polarities (Dhanani et al. 2017). The polarity of a solvent will affect the number of compounds extracted so that it will cause different levels of biological activity generated. The differences in the composition and concentration of these extracted compounds can be evaluated using a metabolomic approach. The metabolomics approach will display a metabolite fingerprinting and/or profiling of the entire metabolite; thus, evaluating the changes in the metabolite profile under various conditions could be performed (Xiao et al. 2012). Metabolomics analysis has been used to determine differences in metabolite profiles due to different extracting solvents, as reported in *Curculigo* sp. (Umar et al. 2021), *Smallanthus sonchifolius* (Aziz et al. 2020), and *Garcinia mangostana* (Mamat et al. 2019). In addition, Rohaeti et al. (2021) have correlated the antioxidant activity of this species based on FTIR spectra. However, information regarding the metabolite profile using UHPLC-Q-Orbitrap HRMS has not been carried out so far.

The extracted metabolite could be identified using the FTIR spectra and UHPLC-Q-Orbitrap HRMS. The FTIR spectra will provide a comprehensive picture of the signals generated by the sample, giving a sample fingerprint because the different compositions and concentrations of each extract will undoubtedly provide a different fingerprint profile. Meanwhile, UHPLC-Q-Orbitrap HRMS will provide information on the previously known chemical compound structure and the overall chemical compound profile. To confirm profile change of the composition and concentration of the compound, multivariate analysis such as Principal Component Analysis (PCA) can be used so that extracts from different solvents will be grouped into their respective groups. In addition, the differences can be proven by testing the free radical scavenging test for DPPH, which is one of the most widely used methods to determine the antioxidant potential of a sample.

This study aims to classify *S. arvensis* extract using three different solvents (water, 50% ethanol, and ethanol p.a.) based on the above explanation. The grouping of metabolites was based on the absorbance spectral data from FTIR and band area data from the UHPLC-Q-Orbitrap HRMS chromatogram combined with PCA. In addition, the free radical scavenging activity was evaluated using the DPPH method to describe the antioxidant potential of each extract. The results of this study provide information related to the standardization process of *S. arvensis* raw materials.

Materials and Methods

Instrument and Software

The instruments used were Fourier transform infrared spectrophotometer (FTIR) Tensor 37 (Bruker Optik GmBH, Ettlingen, Germany), LC-MS/MS Vanquish Flex UHPLC tandem Q Exactive Plus Orbitrap-High Resolution Mass Spectrometer (UHPLC-Q-Orbitrap HRMS) (Thermo Fisher, Waltham, MA, USA), and microplate reader (Epoch-BioTek, Winooski, USA). The software used, namely Thermo XCalibur 2 (Thermo Fisher, Waltham, MA, USA), Compound Discoverer 3.2 (Thermo Fisher, Waltham, MA, USA), and the Unscrambler X version 10.1 (Camo, Oslo, Norway).

Sample Preparation and Extraction

Sonchus arvensis samples were collected from Sine Village, Gondang District, Sragen Regency, Central Java, Indonesia. Identification of the samples was done by Mr. Taufik Ridwan from Tropical Biopharmaca Research Center (TropBRC), IPB University. Voucher specimen (BMK0491062020) was store in TropBRC, IPB University. The samples were macerated with a sample:solvent ratio (1:15) in ethanol p.a., 50% ethanol, and water. The mixtures were soaked for 2×24 h and filtered every 24 h. The filtrate was concentrated using

a rotary evaporator and freeze-dried. The extracts were used for free radical inhibition testing, FTIR spectral preparation, and LC-MS/MS analysis.

DETERMINATION OF FREE RADICAL SCAVENGING ACTIVITY

The extract's free radical scavenging activity was determined using the DPPH method, referring to Salazar-Aranda et al. (2011). A solution of 125 mM DPPH was prepared by weighing 5 mg of DPPH and dissolved in 100 mL ethanol. Sample solutions with a concentration 10000 µg/mL was prepared by weighing 10 mg of extract, dissolved in 1 mL of DMSO, and homogenized using a sonicator. Then, the sample solutions were diluted to a concentration of 100 µg/mL. The test solution consisting of 100 µL of sample solution plus 100 µL of DPPH (125 M in ethanol) was incubated in a dark room at 37 °C for 30 min. The absorbance was then measured at 517 nm in a microplate reader. Vitamin C was used as a positive control, and % inhibition was calculated using the formula:

$$\% \text{ Inhibition} = (1 - (A_{sample} - A_{control}) / (A_{blank} - A_{control}) \times 100\%$$

MEASUREMENT OF FTIR SPECTRUM

Approximately 3 mg of sample extract mixed with 200 mg of KBr was pressed using a hand press for 10 min. Sample fingerprints were analyzed using an FTIR Tensor 37 spectrophotometer (Bruker Optik GmBH, Ettlingen, Germany) equipped with DTGS (deuterated triglycerin sulfate) as the detector. FTIR spectra were made in the middle infrared region (4000-400 cm^{-1}) with a resolution of 4 cm^{-1} and a scan count of 32 s/min operated with OPUS 4.2 software (Bruker Optik GmBH, Ettlingen, Germany).

IDENTIFICATION OF METABOLITES USING UHPLC-Q-ORBITRAP HRMS

A total of 5 mg of the extract was dissolved in 1 mL methanol and sonicated for 30 min. Afterward, the solution was filtered using a PTFE filter with a particle size of 0.2 m. The metabolites were separated using Vanquish Flex UHPLC-Q-Orbitrap HRMS with an Accucore C18 column (100 × 2.1 mm, 1.5 m). The mobile phase used was 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with the following gradient elution system: 0-4 min (10-35% B); 4-30 min (35-95% B), 30-31 min (95% B), 31-32 min (95-20% B), and 32-36 min (20% B). The flow rate used was 0.2 mL/min with an injection volume of 5.0 L. The ionization source used was ESI with positive and negative ionization modes in the *m/z* range of 100-1500. The MS/MS parameters were set under the following conditions: capillary temperature 320 °C, spray voltage 3.8 kV, sheath gas and auxiliary gas 15 and 3 au, respectively, and resolving power of 70,000 FWHM.

UHPLC-Q-Orbitrap HRMS data were processed and analyzed using Compound Discoverer 3.2 to identify the metabolites in the extracts. The metabolites were identified putatively using an in-house database with the following workflows: select spectra, align retention time, detect unknown compounds, group of unknown compounds, predict compositions, search mass list, fill gaps, normalized areas, and marked background compounds.

DATA ANALYSIS

The extracts derived from three different extracting solvents were grouped using PCA multivariate analysis with FTIR absorbance spectra variable at wavenumber 1800-400 cm^{-1} and the peak area of the identified metabolites. In this grouping, preprocessing normalize and standard normal variate for absorbance spectral data of FTIR and center-and-scale for data of the metabolite peak area was carried out using the Unscrambler X version 10.1 software.

Results and Discussion

EXTRACTION YIELD AND FREE RADICAL INHIBITORY ACTIVITY

In this study, we macerated each sample using ethanol, 50% ethanol, and water as solvents. The yield and free radical inhibition are shown in Table 1. Water extract has the highest yield, followed by 50% ethanol and ethanol. The first indication is that the metabolites in *S. arvensis* tend to be polar, which is more soluble in water than in ethanol.

Each extract's free radical scavenging activity was determined at a concentration of 100 µg/mL. The higher the inhibition percentage, the higher the potential of the extract as an antioxidant. Based on the results, 50% ethanol extract gives the highest inhibition (20.61±0.99%), while the aqueous extract gives the lowest inhibitory power (5.87±0.27%). These results are inversely proportional to the extraction yield and indicate that the high extraction yield does not affect the inhibition of free radicals generated. It is likely because the free radical scavenging possessed by the extract is influenced by the presence of phenolic acid or flavonoid which are more soluble in 50% ethanol.

TABLE 1. Extraction yield and free radical scavenging activity of *S. arvensis* extracts

Extract	Yield (%)	Free radical scavenging (%) (100 ppm)
Ethanol	4.74 ± 0.12[a]	6.09 ± 0.29[a]
50% Ethanol	9.01 ± 0.21[b]	20.61 ± 0.99[b]
Water	12.72 ± 1.38[c]	5.87 ± 0.2[7]a

Different letters indicate a significant difference at the 5% confidence level based on Duncan's Test

FTIR SPECTRA OF THE *S. arvensis* EXTRACTS

FTIR spectra were used to identify all metabolite functional groups in the sample. The spectra of the ethanol extract have a slightly different pattern from that of the 50% ethanol and water extracts (Figure 1). These differences show the presence of different functional groups found in the ethanol p.a. extract at wavenumbers 2920 and 2851 cm^{-1}, which indicate the presence of the CH alkanes, and wavenumber 1734 cm^{-1}, which indicates the C=O functional group from aldehyde. Meanwhile, the 50% ethanol and water extracts have similar spectral patterns and differ in their intensity (Figure 1). The difference in intensity can be observed in the fingerprint area's wavenumber of 1600−400 cm^{-1}. The difference in intensity in several absorption bands specifies that different metabolite levels can affect the free radical inhibitory activity caused.

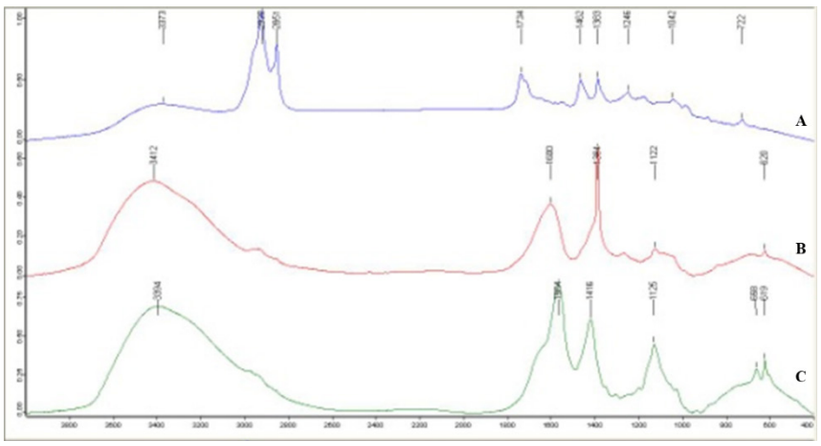


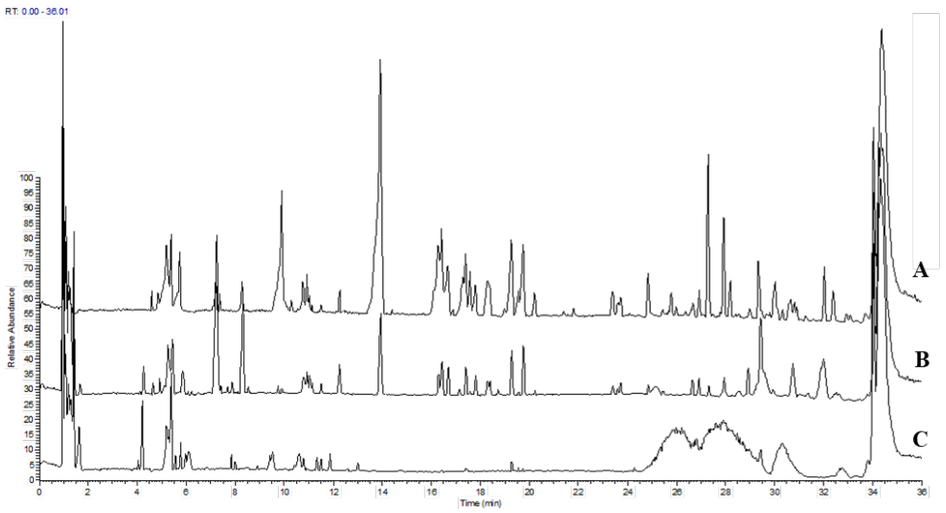
FIGURE 1. FTIR spectra of *S. arvensis* ethanol (a), 50% ethanol (b), and water (c) extract

Table 2 shows the functional groups identified in the *S. arvensis* extract. The absorption band at 3400−3200 cm^{-1} indicates the presence of the OH, which was detected in all samples. In addition, there are other functional groups, such as C=C aromatic at 1600−1475 cm^{-1}, CO at 1300−1000 cm^{-1}, and CH sp^3 at 1450−1375 cm^{-1} detected in FTIR spectra of *S. arvensis* extract.

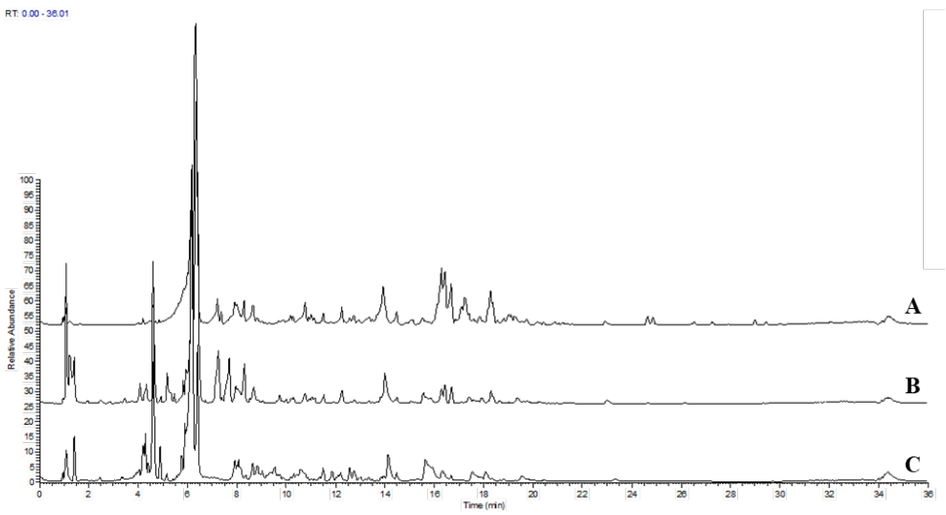
METABOLITE PROFILING OF *S. arvensis* EXTRACTS

The metabolites in the extracts were separated using UHPLC-Q-Orbitrap HRMS. Based on the resulted chromatograms, the ethanol and 50% ethanol extracts have similar chromatogram profiles, indicating similarity in their metabolite composition and content (Figure 2). About 25 were putatively identified from the

chromatograms after MS2 was confirmed using the in-house database (Table 3). Most identified metabolites are in hydroxycinnamic acid, phenolics, and flavonoids groups. The four identified compounds in the hydroxycinnamic acid group are ethyl caffeate, coumaric acid, chicoric acid, and caffeic acid. Caffeic acid was only identified in 50% ethanol extract and fragmented by releasing H_2O and CO_2 molecules at *m/z* 181→163→135.



(a)



(b)

FIGURE 2. Basepeak chromatograms of ethanol (A), 50% ethanol (B), and water (C) extracts of *S. arvensis* in positive (a) and negative (b) ionization modes

Flavonoids are the predominant metabolite present in the extract. In this study, we putatively identified nine compounds from the flavonoid group after confirming using the MS2 spectra (Table 3). Of the several flavonoids compounds, luteolin is the characteristic compound of *S. arvensis*. Luteolin is fragmented in the positive ionization mode to give *m/z* 287 $[M+H]^+$, 153 $[M+H-C_8H_6O_2]^+$, and 135 $[M+H-C_7H_4O_4]^+$ (Table 3). The fragmentation

TABLE 2. Functional groups detected in the *S. arvensis* extracts

Functional groups	Wavenumber (cm^{-1})
OH	3400-3200
CH (Alkanes)	3000-2850
C=O (Aldehydes)	1740-1720
C=C (Aromatics)	1600-1475
CH (CH_3)	1450-1375
CO	1300-1000
CH (Alkenes)	1000-650

TABLE 3. Putatively identified metabolites in *Sonchus arvensis* extracts

No	Metabolite	Formula	MW	Error (ppm)	MS and MS/MS	Ethanol extract	50% ethanol extract	Water extract
1	Vanillin	$C_8H_8O_3$	152.04662	–4.76	151, 136, 123		√	√
2	Vanillic acid hexoside	$C_{14}H_{18}O_9$	330.09552	1.33	329, 151		√	
3	Taraxasterol	$C_{30}H_{50}O$	426.38634	0.41	427, 409, 137	√		
4	Syringic aldehyde	$C_9H_{10}O_4$	182.05737	–2.93	181, 153, 109	√	√	
5	Rhamnetin	$C_{16}H_{12}O_7$	316.05861	0.96	315, 300		√	
6	Protocatechuic acid	$C_7H_6O_4$	154.02594	–4.32	153, 109, 108		√	√
7	Phytol	$C_{20}H_{40}O$	296.30775	–0.55	297, 123, 111, 95, 83, 71	√		
8	p-Coumaroylcaffeoyltartaric acid	$C_{22}H_{18}O_{11}$	458.08532	0.89	457, 295, 163, 149		√	
9	Luteolin-7-O-glucuronide	$C_{21}H_{18}O_{12}$	462.07973	–0.21	461, 285, 133		√	
10	Apigenin glucoronide	$C_{21}H_{18}O_{11}$	446.08496	0.1	447, 271, 153		√	
11	Luteolin-7-glucoside	$C_{21}H_{20}O_{11}$	448.10087	0.68	449, 287, 153	√		
12	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	594.15903	0.95	595, 287, 153		√	
13	Luteolin	$C_{15}H_{10}O_6$	286.04758	–0.54	287, 153, 135	√	√	
14	Hesperetin	$C_{16}H_{14}O_6$	302.07916	0.42	301, 151		√	
15	Ethyl caffeate	$C_{11}H_{12}O_4$	208.07311	–2.17	207, 179, 135	√	√	
16	Esculetin (Dihydroxycoumarin)	$C_9H_6O_4$	178.02615	–2.56	177, 149, 133	√	√	
17	Dihydrophilonotisflavone	$C_{30}H_{20}O_{12}$	572.09569	0.38	571, 285, 151	√	√	
18	Dicaffeoylshikimic acid	$C_{25}H_{22}O_{11}$	498.11663	0.85	499, 163, 145, 135		√	
19	Coumaric acid	$C_9H_8O_3$	164.04730	–0.28	165, 147, 123	√	√	
20	Citric acid	$C_6H_8O_7$	192.02636	–3.33	191, 173, 128, 111		√	
21	Chrysin	$C_{15}H_{10}O_4$	254.05802	0.44	253, 209, 153		√	
22	Chicoric acid	$C_{22}H_{18}O_{12}$	474.08015	0.69	473, 311, 293, 179, 149, 135		√	
23	Caftaric acid	$C_{13}H_{12}O_9$	312.04847	1.1	311, 179, 149, 135		√	
24	Caffeic acid	$C_9H_8O_4$	180.04186	–2.23	181, 163, 145, 135		√	
25	4-Caffeoylquinic acid	$C_{16}H_{18}O_9$	354.09540	0.91	353, 191, 179, 173, 135		√	

occurs due to the cleavage of the flavonoid C ring through the retro-Diels-Alder (RDA) reaction, which is a characteristic of the fragmentation pattern of flavonoid group compounds (Bai et al. 2018). Luteolin is also a compound that plays an important role as an antioxidant with IC_{50} of 2,099 g/mL, 0.59 g/mL, and 0.0402 mmol Fe^{2+}/µg/mL for the DPPH, ABTS, and FRAP methods, respectively (Tian et al. 2021). In this study, luteolin could only be identified in the ethanol p.a. extract and 50% ethanol. It may be due to the very low solubility of luteolin in water, meaning that it cannot be extracted when using the extracting water (Rajhard et al. 2021).

In addition to phenolics and flavonoids, *S. arvensis* also contains terpenoids (Li & Yang et al. 2018). Two terpenoid compounds have been identified in the ethanol p.a. extract, namely taraxasterol and phytol. These two compounds are semipolar, so they have high solubility in ethanol. From the 25 metabolites identified, only two compounds were identified in the aqueous extract, i.e., vanillin and protocatechuic acid. It indicates that most of the compounds identified in *S. arvensis* are semipolar and, therefore, more soluble in ethanol than water. The number of identified metabolites indicates a correlation between free radical inhibitory activity and the metabolites present in the extract. The 50% ethanol extract had the highest inhibitory activity and contained more metabolites than the ethanol and water extracts (Table 3).

CLUSTERING OF *S. arvensis* EXTRACTS

All of the *S. arvensis* extracts were showed similar FTIR spectra and UHPLC-Q-Orbitrap HRMS chromatograms profiles. Therefore, chemometric analysis, such as PCA, is required to cluster the sample extracts based on their extraction solvent. The PCA is an unsupervised multivariate analysis that will visualize the grouping of samples based on the proximity of their metabolite composition. In addition, the PCA can reduce data and extract information to find combinations of variables that describe the data set (Ghallab et al. 2020). In this study, both the PCA models were made using absorbance at 1800−400 cm^{-1} and the peak area of the identified metabolites as a variable.

The PCA score plot is shown in Figure 3(a) using absorbance data from the FTIR spectra and Figure 3(b)

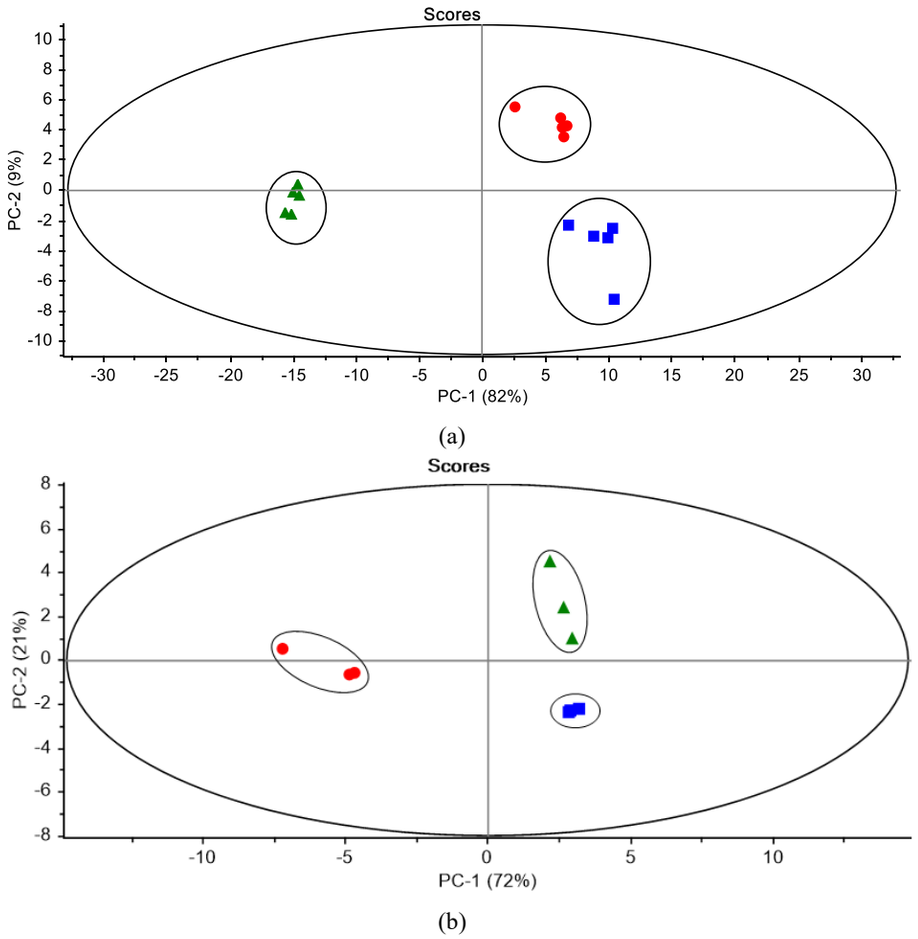


FIGURE 3. PCA plot scores of FTIR spectra (a) and the identified metabolites (b) of *S. arvensis* extracts (green: ethanol extract, red: 50% ethanol extract, blue: water extract)

using peak area of identified metabolites as the variable. The resulting score plot shows the groups of extracts based on the differences in the extracting solvents. Both score plots successfully grouped the extracts using two PCs with a total PC of 91% for plotting the FTIR spectra score and 93% for plotting the metabolite peak area score. Adjacent groups showed a high similarity of metabolite profiles. The PCA with FTIR spectra data shows a closeness between the 50% ethanol and the water extracts, meaning a similarity in the FTIR spectra fingerprints.

Meanwhile, using the variable peak area of identified metabolites, aqueous extracts tend to be close to the ethanol extract. The grouping of extracts using FTIR spectra data and the peak area of the metabolites showed a clear separation, meaning that each sample could be distinguished using either the FTIR-spectra fingerprint or the peak area of identified metabolites from UHPLC-Q-Orbitrap HRMS analysis.

Conclusion

Sonchus arvensis extracts with different extracting solvents give different free radical inhibitory activities. The 50% ethanol extract exhibited the highest free radical inhibition than the other two, but similar FTIR fingerprints and UHPLC-MS/MS chromatograms. A total of 25 metabolites were putatively identified in the extract, most of which belong to the phenolics and flavonoids. Clustering *S. arvensis* extracts with different extracting solvents were successfully obtained using the FTIR spectra and peak area of the identified metabolites combined with PCA. We conclude that the extracting solvent affected the composition of the metabolites in the sowthistle, resulting in different free radical inhibitory activity levels.

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References

Aziz, Z., Yuliana, N.D., Simanjuntak, P., Rafi, M. & Abdillah, S. 2020. FTIR and HPLC-based metabolomics of yacon leaves extracts (*Smallanthus sonchifolius* [Poepp & Endl.] H. Robinson) from two locations in Indonesia. *Indonesian Journal of Chemistry* 20(3): 567-578. doi: 10.22146/ijc.43453

Bai, Y., Zheng, Y., Pang, W., Peng, W., Wu, H., Yao, H., Li, P., Deng, W., Cheng, J. & Su, W. 2018. Identification and comparison of constituents of Aurantii Fructus and Aurantii Fructus Immaturus by UFLC-DAD-Triple TOF-MS/MS. *Molecules* 23(4): 803. doi: 10.3390/molecules23040803.

Dhanani, T., Shah, S., Gajbhiye, N.A. & Kumar, S. 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry* 10(1): S1193-S1199. doi: 10.1016/j.arabjc.2013.02.015.

Dutta, K.N., Lahkar, M., Sarma, D. & Sharma, R.K. 2020. A comparative study on the antidiabetic activity of *Sonchus asper* and *Sonchus arvensis* in alloxan induced diabetic rats. *International Journal of Scientific Development and Research* 5(5): 1-15.

Ghallab, D.S., Mohyeldin, M.M., Shawky, E., Metwally, A.M. & Ibrahim, R.S. 2020. Chemical profiling of Egyptian propolis and determination of its xanthine oxidase inhibitory properties using UPLC–MS/MS and chemometrics. *LWT-Food Science and Technology* 136: 1-16. doi:10.1016/j.lwt.2020.110298.

Kanaani, S. & Sani, A.M. 2015. Chemical composition of essential oils and *in vitro* antibacterial activity of methanolic extract of *Sonchus arvensis* and *Eremurus spectabilis* against food-borne pathogenic bacteria. *Journal of Essential Oil-Bearing Plants* 18(5): 1093-1099. doi:10.1080/0972060X.201.

Karamian, K. & Asadbegy, M. 2016. Antioxidant activity, total phenolic and flavonoid contents of three Onobrychis species from Iran. *Pharmaceutical Sciences* 22: 112-119. doi: 10.15171/PS.2016.18.

Khan, R.A. 2012. Evaluation of flavonoids and diverse antioxidant activities of *Sonchus arvensis*. *Chemistry Central Journal* 6: 126. doi:10.1186/1752-153X-6-126

Li, X.M. & Yang, P.L. 2018. Research progress of Sonchus species. *International Journal of Food Properties* 21(1): 147-157. doi:10.1080/10942912.2017.1415931.

Mamat, S.F., Azizan, K.A., Baharum, S.N., Mohd-Nor, N. & Aizat, W.M. 2019. The influence of two solvent extraction ratios in GC-MS-based metabolomics of different *Garcinia mangostana* Linn. fruit tissues. *International Food Research Journal* 26(5): 1447-1457.

Prasad, S.J., Kumar, P.B., Raj, S.S. & Sadhana, A. 2015. Hypoglycemic and analgesic effects of methanolic extracts of *Sonchus arvensis* from Nepal. *International Journal of Pharmacognosy and Phytochemical Research* 7(3): 613-615.

Rajhard, S., Hladnik, L., Vicente, F.A., Srčič, S., Grilc, M. & Likozar, B. 2021. Solubility of luteolin and other polyphenolic compounds in water, nonpolar, polar aprotic and protic solvents by applying FTIR/HPLC. *Processes* 9(11): 1952. doi: 10.3390/pr9111952

Rohaeti, E., Karunina, F. & Rafi, M. 2021. FTIR-based fingerprinting and chemometrics for rapid investigation of antioxidant activity from *Syzygium polyanthum* extracts. *Indonesian Journal of Chemistry* 21(1): 128.

Salazar-Aranda, R., Pérez-López, L.A., López-Arroyo, J., Alanís-Garza, B.A. & Waksman de Torres, N. 2011. Antimicrobial and antioxidant activities of plants from northeast of Mexico. *Evid. Based Complement Alternat. Med*. 2011: 536139. doi:10.1093/ecam/nep127.

Sukandar, E.Y., Garmana, A.N., Aidasari, A.U. & Crystalia, A.A. 2019. Antihypertensive activity of ethanol extract combination of *Anredera cordifolia* (Ten.) v. Steenis and *Sonchus arvensis* L. leaves on angiotensin II-induced male wistar rat. *Journal of Research in Pharmacy* 23(6): 1090-1097. doi: 10.35333/jrp.2019.73.

Tian, C., Liu, X., Chang, Y., Wang, R., Lv, T., Cui, C. & Liu, M. 2021. Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. *South African Journal of Botany* 137: 257-264. doi:10.1016/j.sajb.2020.10.022.

Umar, A.H., Ratnadewi, D., Rafi, M. & Sulistyaningsih, Y.C. 2021. Untargeted metabolomics analysis using FTIR and UHPLC-Q-Orbitrap HRMS of two *Curculigo species* and evaluation of their antioxidant and -glucosidase inhibitory activities. *Metabolites* 11(1): 42. doi:10.3390/metabo11010042.

Widyarini, K.D., Sukandar, E.Y. & Fidrianny, I. 2015. Xanthine oxidase inhibitory and antihyperuricemic activities of *Anredera cordifolia* (Ten) Steenis, *Sonchus arvensis* L., and its combination. *International Journal of Pharmacy and Pharmaceutical Sciences* 7(3): 86-90.

Xiao, J.F., Zhou, B. & Ressom, H.W. 2012. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. *Trends in Anaytical Chemistry* 32: 1-14. doi: 10.1016/j.trac.2011.08.009.

*Corresponding author; email: mra@apps.ipb.ac.id