BUKTI KORESPONDENSI
ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul : Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract
Jurnal : Food Research **(Scopus Q3)**
Penulis : Herpandi, Lestari, S.D., Bastian and ***Sudirman, S.**
Kontribusi : **Penulis Korespondensi**

No.	Perihal	Tanggal
1.	Bukti submit dan konfirmasi submit artikel	Oktober 2020
2.	Manuscript 1st revision	14 Nov 2020
3.	Manuscript accepted	18 Jan 2021
4.	Galley proof and invoice	25 April 2021
5.	Invoice payment	26 April 2021
6.	Article published	29 April 2021

Bukti submit dan konfirmasi submit

Sabri Sudirman UNSRI <sabrisudirman@unsri.ac.id>
to Food

Sun, 11 Oct 2020, 10:05

Dear,
Vivian New Chia Yeung
Chief Editor
Food Research

We are pleased to submit an original research article entitled "Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract" for consideration for publication in the Food Research journal.

In this experiment, we found that the bioactive compound from water lettuce (Pistia stratiotes) successfully extracted by methanol solvent with a high yield of extraction. The crude methanol extract was separated by thin-layer chromatography and column chromatography and there are seven fractions. Whereas, the third fraction showed more high antioxidant activity. Also, this fraction showed a high hydroxyl group. The hydroxyl group of the polyphenols showed a positive correlation with their antioxidant activity.

We believe that this manuscript is appropriate for publication by the Food Research journal because it is suitable for the journal's aims and scope. This manuscript has not been published and is not under consideration for publication elsewhere.

Thank you for your consideration.

Sincerely,
Sabri Sudirman, Ph.D.
Fisheries Product Technology
Faculty of Agriculture, Sriwijaya University, Indonesia
http://www.fp.unsri.ac.id
Department of Food Science
National Taiwan Ocean University, Keelung City, Taiwan
http://www.fs.ntou.edu.tw
ResearcherID: O-2980-2018
Scopus ID: 56513896600
ORCID: 0000-0003-2821-3772
e-mail: sabrisudirman@unsri.ac.id

3 Attachments • Scanned by Gmail

Manuscript ID: FR-2020-578 Inbox ×

Food Research <foodresearch.my@outlook.com>
to me

Sun, 11 Oct 2020, 18:06

Dear Dr. Sabri Sudirman,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,

Professor Dr. Son Radu
Chief Editor
Email: foodresearch.my@outlook.com

11th October 2020

Dear,
Vivian New Chia Yeung
Chief Editor
Food Research
foodresearch.my@outlook.com

We are pleased to submit an original research article entitled "Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract" for consideration for publication in the Food Research journal.

In this experiment, we found that the bioactive compound from water lettuce (*Pistia stratiotes*) successfully extracted by methanol solvent with a high yield of extraction. The crude methanol extract was separated by thin-layer chromatography and column chromatography and there are seven fractions. Whereas, the third fraction showed more high antioxidant activity. Also, this fraction showed a high hydroxyl group. The hydroxyl group of the polyphenols showed a positive correlation with their antioxidant activity.

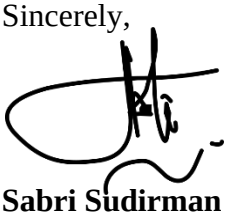
The potential reviewers for this manuscript:

1. Ms. Erica Souto Abreu Lima. Water lettuce. ericaabreulima@gmail.com
2. Mr. Chinedu Anokwuru. Antioxidant. anokwuruc@babcockuni.edu.ng
3. Mr. Mohd Mujeeb. Extraction method. drmmujeeb12@gmail.com
4. Mr. Zübeyir Huyut. Bioactive compound. zubeyir.huyut@gmail.com

We believe that this manuscript is appropriate for publication by the Food Research journal because it is suitable for the journal's aims and scope. This manuscript has not been published and is not under consideration for publication elsewhere.

Thank you for your consideration.

Sincerely,



Sabri Sudirman
Corresponding author
Fisheries Product Technology
Faculty of Agriculture
Universitas Sriwijaya
E-mail: sabrisudirman@.unsri.ac.id

Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

Herpandi, Lestari, S.D., Bastian, *Sudirman, S.

Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya, Ogan Ilir Regency 30862, South Sumatra, Indonesia

*Corresponding author: sabrisudirman@unsri.ac.id

Author No.1: ORCID 0000-0002-2186-7653

Author No.2: ORCID 0000-0002-5718-8513

Author No.3: ORCID 0000-0002-5875-5663

Author No.4: ORCID 0000-0003-2821-3772

Abstract

Reactive oxygen species and free radicals are continuous increases in the human body. Oxidative stress is the unbalancing condition with a high ratio of free radicals compared to antioxidants. An antioxidant is a compound with the ability to reduce the harmfulness of the free radical. This study aimed to determine the antioxidant activity of fractions and analyzed the functional groups of water lettuce (*Pistia stratiotes*) methanol extract. The separation process was performed by using thin-layer chromatography (TLC) and column chromatography. The separated-fractions was measured the antioxidant activity by using the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The functional groups of each fraction were determined by using Fourier-transform infrared (FT-IR) spectroscopy. The separation of water lettuce extract by using column chromatography produced seven fractions with

different colors and confirmed by using TLC. The antioxidant activity showed the highest activity in the third fraction with a half-maximal inhibitory concentration (IC_{50}) value of 131.66 ppm. The fifth fraction with the IC_{50} was about 184.62 ppm. Whereas, in the first, second, fourth, sixth, and seventh fractions relatively very weak with the IC_{50} more than 200 ppm. The FT-IR spectrum also showed that the intensity hydroxyl group in the third fraction higher than the seventh fraction.

Keywords: Antioxidant, free radicals, hydroxyl group*, Pistia stratiotes*, separation

1. Introduction

Reactive oxygen species (ROS) and free radicals, such as anion superoxide (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are continuous increases in the human body (Phaniendra *et al.*, 2014). The high level of free radicals compared to antioxidants leading to oxidative stress conditions. This condition is involved in some chronic and low-inflammation diseases, such as insulin resistance in type-2 diabetes, rheumatoid arthritis, cardiovascular diseases, and aging disease (Khansari *et al.*, 2009). Therefore, the body needs exogenous antioxidants through functional food products, fruits, vegetables, and food supplements (Bouayed and Bohn, 2010).

An antioxidant is a compound with the ability to reduce the harmfulness of the free radicals (Lobo *et al.*, 2010). According to its source, the antioxidant can be divided into two groups include endogenous (primary) antioxidants, such as superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPx), whereas the second group is exogenous (secondary) antioxidants. This type of antioxidant can get from the diet by eating antioxidant-rich foods or food supplements (Bouayed and Bohn, 2010). Water lettuce is one of the aquatic plants which possess antioxidant properties. This plant also contains some bioactive compounds, such as polyphenols, flavonoids, and saponin (Sudirman *et al.*, 2017a; Sudirman *et al.*, 2017b). According to the previous study, water lettuce (*Pistia stratiotes*) methanol extract showed

high antioxidant activity when compared to *n*-hexane and ethyl acetate extracts. This extract is composed of polyphenols and flavonoids (Sudirman *et al.*, 2017a). A previous study reported polyphenols reduced the harmfulness of the free radical by transferring the hydrogen (H) atom from their hydroxyl (OH) groups (Foti, 2007).

However, in the previous study, the authors still used a crude extract of the water lettuce. Therefore, in the present study, we tried to separate the methanol crude extract by using thin-layer chromatography (TLC) and column chromatography. The TLC and column chromatography methods have been widely used to separate plant extract to several fractions (Kagan and Flythe, 2014). A previous study reported that different fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019). According to these conditions, we hypothesized that the antioxidant activity of the water lettuce methanol extract will be increased after the separation process. Therefore, this study aimed to investigate the antioxidant activity of methanol fraction from water lettuce (*Pistia stratiotes*) after separation and confirm their hydroxyl group by Fourier-transform infrared.

2. Materials and methods

2.1 Water lettuce extraction

The fresh form of water lettuce (*Pistia stratiotes*) was collected at swampy waters in Palembang city, South Sumatera, Indonesia, and cleaned to remove unwanted-materials. Then, it was reduced the size, dried, and extract by the following previous method (Sudirman *et al.*, 2017b). Briefly, 20 g of dried sample was extracted by using 200 mL of methanol (1:10, v/v) at room temperature for 24 h. The solution was filtrated by using a filter paper, solvent removed by using a rotary vacuum evaporator, and dried by using an oven dryer. The sample was kept for future analysis. The percent of extraction yield was

calculated from the weight of dried extract divided by the weight of the dried sample and multiplied by 100%.

2.2 Separation Process

The thin-layer chromatography (TLC) and column chromatography methods have been widely used to separate fractions from plant extracts. The silica TLC plate and silica gel were purchased from Merck KGaA (Darmstadt, Germany). The TLC method in this research followed the previous study (Kagan and Flythe, 2014). Briefly, the plant extract was dissolved in methanol to have a sample concentration. The TLC plate was cut in a piece (2 cm x 10 cm) and leaving a 1 cm border on the upper- and bottom-sides of the plate. The sample was loaded by using a microliter syringe on the plate band and allow to dry. The plate with the sample was then developed in the cover chamber which containing mixed solvents or eluent (methanol-ethyl acetate-acetone 1:1:1, v/v/v, for this study). After the developing step, the plate was dried and observed bands under visible or UV light (254 nm and 365 nm) then marked bands with a pencil. The mixed solvent was used to further separation by using silica gel column chromatography. Whereas, the column chromatography was performed by the following previous method (Venkatesh *et al.*, 2017). The mixed solvent was loaded into the packed silica column. The seven different separated-fractions (confirmed by TLC) were collected, dried, and kept for future analysis.

2.3. Antioxidant activity assay

The antioxidant activity assay was performed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by the previous method (Molyneux, 2004). The DPPH powder was purchased from Merck KGaA (Darmstadt, Germany). Briefly, each of the dried-fractions was dissolved in a methanol solvent to make a serial concentration (50 ppm, 100 ppm, 150 ppm, and 200 ppm). Whereas, Vitamin C (containing ascorbic acid) was used as a positive control. A hundred sixty (160) µL of dried-fraction was added into a well which containing 40 µL of 0.76 mM of DPPH (10 mg DPPH in 10 mL of methanol). The solution was incubated at 37°C for 30 min. after incubation time, the absorbance was immediately measured at 517

nm by using spectrophotometry. The percent of inhibition (%) was calculated from absorbance of blank minus absorbance of the sample divided by absorbance of blank and multiplied by 100%.

2.4. Functional group analysis

The functional group was analyzed by using a Fourier transform-infrared (FT-IR) spectroscopy according to the previous methods (Ouhaddouch *et al.*, 2019). Briefly, the sample was placed into the infrared beam (sample holder), then measured in the spectral range between 500 cm^{-1} and 4000 cm^{-1}. Whereas, KBr-pressed disk was used in the FT-IR spectroscopy (Spectrum One Perkin Elmer, Massachusetts, USA).

3. Results and Discussion

This study demonstrated the antioxidant activity of the fractions from the Water lettuce (*Pistia stratiotes*) methanol extract. The previous studies reported that the methanol extract is composed of polyphenols, such as flavonoids and tannin (Sudirman *et al.*, 2017a; Sudirman *et al.*, 2017b). These studies also reported that methanol extract showed the highest antioxidant activity compared to *n*-hexane and ethyl acetate extracts. In addition, methanol extract also shows a high yield of the extract (Benhammou *et al.*, 2009). Therefore, this present study only separated the methanol extract from water lettuce to some fractions. The yield of water lettuce methanol extract was about 16.16%. A previous study also reported that methanol is the best solvent for the extraction of leaves of *Acalypha wilkesiana* and *Atriplex halimus* were about 14.67% and 24.00%, respectively (Benhammou *et al.*, 2009; Anokwuru *et al.*, 2016). Methanol has been widely used for bioactive extraction from the plant. Methanol is also more efficient in the extraction of polyphenols especially low molecular weight (MW) of polyphenols (Do *et al.*, 2014).

The fractions from methanol extract were separated by using thin-layer chromatography (TLC) and column chromatography methods. Based on the TLC results, we found that the best-mixed solvent is

114 composed of methanol, ethyl acetate, and acetone with the ratio 1:1:1, as shown in **Figure 1A**. According
115 to this result, this eluent was used for the mobile phase in column chromatography. After separation by
116 column chromatography, the result showed that there are seven fractions of the methanol extracts as
117 shown in **Figure 1B**. The TLC and column chromatography methods have been widely used to separate
118 plant extract to several fractions (Kagan and Flythe, 2014).

119 The antioxidant activity of the fraction was evaluated by using the 1,1-diphenyl-2-picrylhydrazyl
120 (DPPH) method. The DPPH method was widely used to measure the antioxidant activity of the extract due
121 to its speed, simplicity, and low cost (Alam *et al.*, 2013). As shown in **Table 1**, third (F3) and fifth (F5)
122 fractions possessed highly antioxidant activities with the half-maximum inhibitory concentration (IC_{50})
123 was about 131.66 ppm and 184.62 ppm, respectively. Whereas, other fractions showed weak antioxidant
124 activities with the IC_{50} more than 200 ppm (Molyneux, 2004). A previous study reported that IC_{50} of crude
125 methanol extract of water lettuce was about 147.60 ppm (Sudirman *et al.*, 2017a). The different fractions
126 in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*,
127 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*,
128 2019).

129 According to the antioxidant activity assay, the highest activity (third, F3) fraction was continued
130 for analysis of its functional group by using Fourier transform-infrared (FT-IR) spectroscopy then
131 compared to the seventh fraction (F7) as a representative from the fraction of weak antioxidant activity
132 as shown in **Figure 2** and **Table 2**. A previous study reported that different bounds of the molecule also
133 show different vibrational frequencies of FTIR spectroscopy, such as C–C, C=C, C–O, C=O, O–H, and N–H
134 bonds (Altemimi *et al.*, 2017). The functional group of each wavenumber in **Table 2** was evaluated
135 according to the previous reference (Ouhaddouch *et al.*, 2019). **Figure 2** showed that the O-H bond in F3
136 fraction (3432.90 cm^{-1}) more than F7 fraction (3366.06 cm^{-1}). The number of OH groups bound to aromatic

ring is positively correlative with the antioxidant activity of polyphenols (Zielinska-Blizniewska *et al.*, 2019).

4. Conclusion

Overall, the bioactive compound from water lettuce (*Pistia stratiotes*) was successfully extracted by methanol solvent. The methanol extract showed seven fractions after separation by thin-layer chromatography and column chromatography. Whereas, the third fraction possessed high antioxidant activity. The Fourier transform-infrared confirmed that the hydroxyl group of the polyphenols play an important role in their antioxidant activity.

Conflict of interest.

The authors declare no conflict of interest.

Acknowledgments

We are thanks to the Indonesian Ministry of Education and Culture to support this study.

References

Alam, M.N., Bristi, N.J., Rafiquzzaman, M., 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal 21, 143-152.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., Lightfoot, D., 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants 6, 42.

158 Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O., Okebugwu, P., 2016. Effect of Extraction Solvents
159 on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants. Nature and
160 Science 9, 53-61.
161 Benhammou, N., Bekkara, F.A., Kadifkova Panovska, T., 2009. Antioxidant activity of methanolic extracts
162 and some bioactive compounds of Atriplex halimus. Comptes Rendus Chimie 12, 1259-1266.
163 Bouayed, J., Bohn, T., 2010. Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State:
164 Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses. Oxidative
165 Medicine and Cellular Longevity 3, 228-237.
166 Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., Ju, Y.-H., 2014.
167 Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity
168 of Limnophila aromatica. Journal of Food and Drug Analysis 22, 296-302.
169 Foti, M.C., 2007. Antioxidant properties of phenols. Journal of Pharmacy and Pharmacology 59, 1673-
170 1685.
171 Kagan, I.A., Flythe, M.D., 2014. Thin-layer Chromatographic (TLC) Separations and Bioassays of Plant
172 Extracts to Identify Antimicrobial Compounds. Journal of Visualized Experiments 85, e51411.
173 Khansari, N., Shakiba, Y., Mahmoudi, M., 2009. Chronic Inflammation and Oxidative Stress as a Major
174 Cause of Age- Related Diseases and Cancer. Recent Patents on Inflammation & Allergy Drug
175 Discovery 3, 73-80.
176 Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact
177 on human health. Pharmacognosy Reviews 4, 118.
178 Molyneux, P., 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating
179 antioxidant activity. Songklanakarin Journal of Science and Technology (SJST) 26, 211-219.

Ouhaddouch, H., Cheikh, A., Idrissi, M.O.B., Draoui, M., Bouatia, M., 2019. FT-IR Spectroscopy Applied for Identification of a Mineral Drug Substance in Drug Products: Application to Bentonite. Journal of Spectroscopy 2019, 1-6.

Phaniendra, A., Jestadi, D.B., Periyasamy, L., 2014. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian Journal of Clinical Biochemistry 30, 11-26.

Sudirman, S., Herpandi, H., Lestari, S.D., Wasahla, W., 2017a. Phytochemicals Screening and Antioxidant Activity of Water Lettuce (*Pistia Stratiotes*) Extract. European Journal of Scientific Research 146, 234-238.

Sudirman, S., Herpandi, H., Nopianti, R., Dwita Lestari, S., Wasahla, W., Mareta, H., 2017b. Phenolic Contents, Tannin, Vitamin C, and Vitamin E of Water Lettuce (Pistia Stratiotes). Oriental Journal of Chemistry 33, 3173-3176.

Triadisti, N., Sauriasari, R., Elya, B., 2018. Antioxidant Activity of Fractions from Garcinia hombroniana Pierre Leaves Extracts. Pharmacognosy Journal 10, 682-685.

Venkatesh, U., Javarasetty, C., Murari, S.K., 2017. Purification and Fractional Analysis of Methanolic Extract of Wedelia Trilobata Possessing Apoptotic and Anti-Leukemic Activity. African Journal of Traditional, Complementary and Alternative medicines 14, 167-174.

Zhao, Q.-C., Zhao, J.-Y., Ahn, D.U., Jin, Y.-G., Huang, X., 2019. Separation and Identification of Highly Efficient Antioxidant Peptides from Eggshell Membrane. Antioxidants 8, 495.

Zielinska-Blizniewska, H., Sitarek, P., Merecz-Sadowska, A., Malinowska, K., Zajdel, K., Jablonska, M., Sliwinski, T., Zajdel, R., 2019. Plant Extracts and Reactive Oxygen Species as Two Counteracting Agents with Anti- and Pro-Obesity Properties. International Journal of Molecular Sciences 20, 4556.

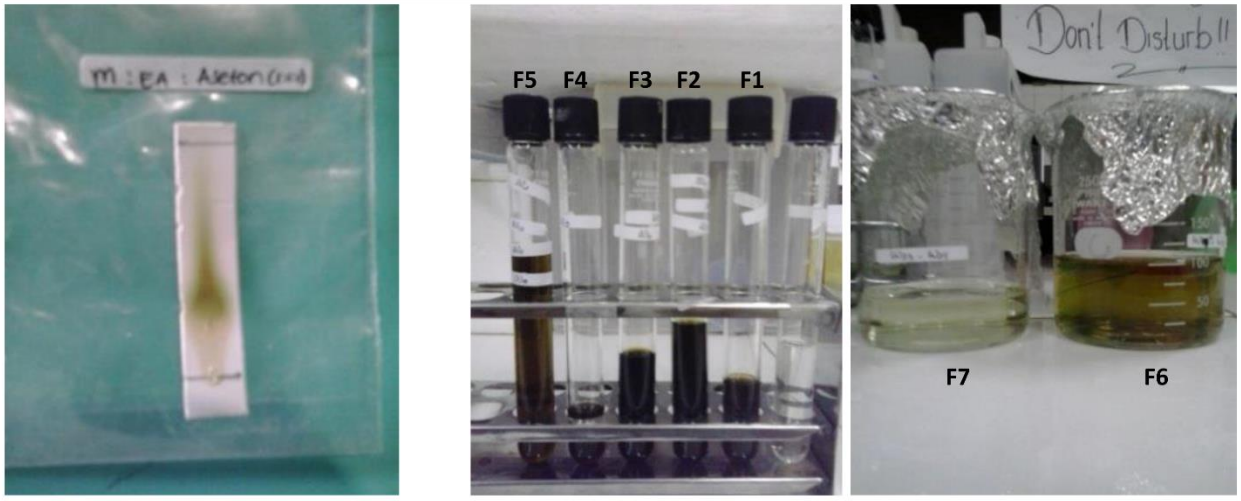


Figure 1. Methanol extract separation: **(A)** Thin-layer chromatography and **(B)** The separated-fractions after the separation by using column chromatography.

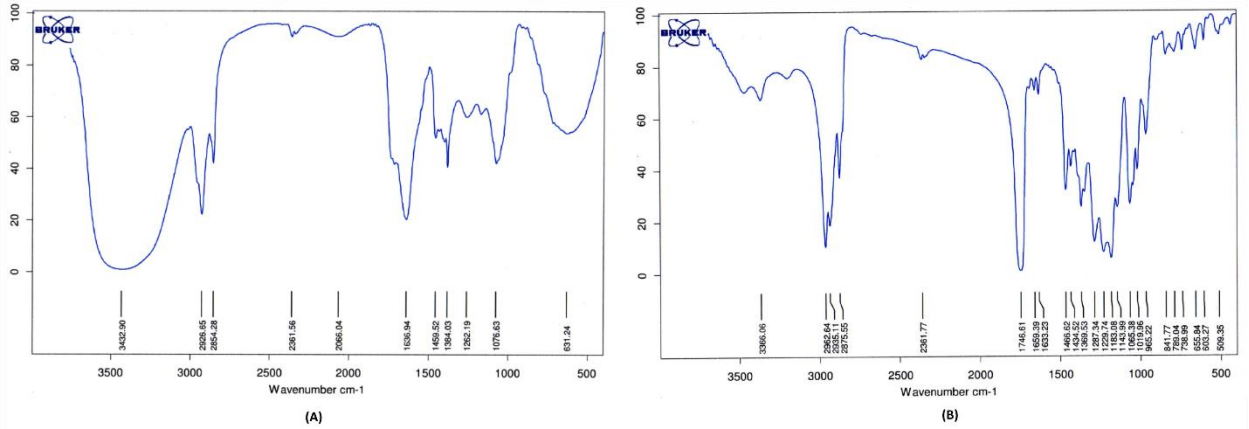


Figure 2. The Fourier transform-infrared (FT-IR) spectrums of the **(A)** Third fraction, F3 and **(B)** Seventh fraction, F7.

208 **Table 1**. The antioxidant activity of the fractions.

Sample	F1	F2	F3	F4	F5	F6	F7	VC
IC_{50} (ppm)	642.97	203.90	131.66	459.61	184.62	>2000	>2000	4.14

209 Abbreviations: F, sample fraction; IC_{50}, Half-maximum inhibitory concentration; VC, vitamin C as a positive control.

210

211 **Table 2.** The functional groups of third fraction and seventh fraction.

Sample	Wavenumber (cm^{-1})	Wavenumber reference (cm^{-1})	Functional groups
Third Fraction (F3)	3432.90	3200-3500	O-H
	2926.65	2800-3000	C-H
	2854.28		
	1636.94	1560-1640	N-H
	1459.52	1400-1500	C-H
	1076.63	1020-1250	C-N, C-O, C-C
Seventh fraction (F7)	3366.06	3200-3500	O-H
	2962.64	2800-3000	C-H
	2935.11		
	2875.55		
	1659.39	1560-1640	N-H
	1633.23		
	1466.62	1400-1500	C-H
	1434.52		
	1065.38	1020-1250	C-N, C-O, C-C
	1019.96		

212

FOOD RESEARCH

MANUSCRIPT SUBMISSION FORM

Please fill in your manuscript details in **'New Manuscript Submission Section'** and submit this form together with your manuscript.
Authors are requested to suggest at least 3 – 4 potential reviewers by filling in their particulars in the **'Suggested Reviewer'** section.

NEW MANUSCRIPT SUBMISSION

Manuscript Title	Antioxidant activity of the fractions from water Lettuce (*Pistia stratiotes*) extract
Manuscript Type *(Please Bold)*	**Original Article** Review Short Communication Technical Notes
Authors	Herpandi; Lestari, S.D.; Bastian; Sudirman, S.
Corresponding Author *(Only one)*	Sudirman, S.
Email address of the Corresponding Author	sabrisudirman@unsri.ac.id

SUGGESTED REVIEWERS

Name	Erica Souto Abreu Lima
Salutation	Ms.
Area of Expertise *(Please use comma for more than one)*	Water lettuce
Email	ericaabreulima@gmail.com

Name	Chinedu Anokwuru
Salutation	Mr.
Area of Expertise *(Please use comma for more than one)*	Antioxidant
Email	anokwuruc@babcockuni.edu.ng

Name	Mohd Mujeeb
Salutation	Mr.
Area of Expertise *(Please use comma for more than one)*	Extraction method
Email	drmmujeeb12@gmail.com

Name	Zübeyir Huyut
Salutation	Mr.
Area of Expertise *(Please use comma for more than one)*	Bioactive compound
Email	zubeyir.huyut@gmail.com

FOOD
RESEARCH

10th October 2020

Authors: Herpandi, Lestari, S.D., Bastian, Sudirman, S.

Manuscript title: Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

Manuscript ID: FR-2020-578

Dear Dr. Sabri Sudirman,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,

Professor Dr. Son Radu
Chief Editor
Email: foodresearch.my@outlook.com

Manuscript 1st revision

Food Research <foodresearch.my@outlook.com>
to me

Wed, 11 Nov 2020, 00:27

Dear Dr. Sabri Sudirman,

Manuscript FR-2020-578 entitled " Antioxidant activity of the fractions from water lettuce (Pistia stratiotes) extract " which you submitted to Food Research, has been reviewed. The comments of the reviewer(s) are included in the attached file.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Once the revised manuscript is prepared, please send it back to me for further processing.

Because we are trying to facilitate timely publication of manuscripts submitted to Food Research, your revised manuscript should be submitted before or by 21st November 2020. If it is not possible for you to submit your revision by this date, please let us know.

Once again, thank you for submitting your manuscript to Food Research and I look forward to receiving your revised manuscript.

Sincerely,
Professor Dr. Son Radu
foodresearch.my@outlook.com

Chief Editor, Food Research

From: Food Research <foodresearch.my@outlook.com>
Sent: Sunday, 11 October, 2020 7:05 PM
To: Sabri Sudirman UNSRI <sabrisudirman@unsri.ac.id>
Subject: Manuscript ID: FR-2020-578

Sabri Sudirman UNSRI <sabrisudirman@unsri.ac.id>
to Food

Sat, 14 Nov 2020, 19:40

Dear,
Professor Dr. Son Radu
Editor-in-Chief
Food Research

Manuscript ID: **FR-2020-578**
Title: Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

According to reviewer comments, we have revised our manuscript.
The manuscript revision is attached.

Thank you for the comments. We hope this revision can help our manuscript to publish in the Food research journal.
Thank you for your consideration.

Sincerely,
Sabri Sudirman
•••
--
•••

3 Attachments • Scanned by Gmail

Food Research <foodresearch.my@outlook.com>
to me

14 Nov 2020, 23:27

Dear Dr. Sabri Sudirman,

Thank you for the revised copy of your manuscript. We will contact you again soon for further processing.

Best regards,
Son Radu, PhD
Chief Editor

From: Sabri Sudirman UNSRI <sabrisudirman@unsri.ac.id>
Sent: Saturday, 14 November, 2020 8:40 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: Manuscript ID: FR-2020-578

•••
...

[Message clipped] View entire message

14th November 2020

Dear,
Professor Dr. Son Radu
Chief Editor
Food Research
foodresearch.my@outlook.com

Manuscript ID: **FR-2020-578**
Title: Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

According to reviewer comments, we have revised our manuscript.

Reviewer comments:

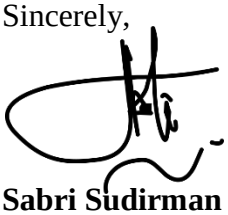
1. Manuscript format
2. References: Reference style and species name

Response:

- We have revised the manuscript format and references by following the Food Research guidelines.
- The species name also in Italics font style.

Thank you for the comments. We hope this revision can help our manuscript to publish in Food research journal.
Thank you for your consideration.

Sincerely,



Sabri Sudirman
Corresponding author
Fisheries Product Technology
Faculty of Agriculture
Universitas Sriwijaya
E-mail: sabrisudirman@.unsri.ac.id

Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

Herpandi, Lestari, S.D., Bastian and *Sudirman, S.

Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya, Ogan Ilir Regency 30862, South Sumatra, Indonesia

*Corresponding author: sabrisudirman@unsri.ac.id

Article history:

Received: 9 October 2020

Received in revised form: 14 November 2020

Accepted: 18 January 2021

Abstract

Free radicals including reactive oxygen species are continuous increases in the human body. This condition causes unbalancing between free radicals and antioxidants. An antioxidant is a compound with the ability to reduce the harmfulness of the free radical. This study aimed to determine the antioxidant activity of fractions and analyzed the functional groups of water lettuce (*Pistia stratiotes*) methanol extract. The separation process was performed by using thin-layer chromatography (TLC) and column chromatography. The separated fractions were measured for their antioxidant activity by using the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The functional groups of each fraction were determined by using Fourier-transform infrared (FT-IR) spectroscopy. The separation of water lettuce extract by using column chromatography produced seven fractions with different colors and confirmed by using TLC. The antioxidant activity showed the highest activity in the third fraction with a half-maximal inhibitory concentration (IC_{50}) value of 131.66 ppm. The fifth fraction with the IC_{50} was about 184.62 ppm. Whereas, the first, second, fourth, sixth, and seventh fractions were relatively weak with the IC_{50} more than 200 ppm. The FT-IR spectrum also showed that the intensity hydroxyl group in the third fraction higher than the seventh fraction.

Keywords: Antioxidant, Free radicals, Hydroxyl group*, Pistia stratiotes*, Separation

Commented [G1]: Please rephrase. The sentence is confusing.

Commented [SS2R1]: Free radicals including reactive oxygen species are continuous increases in the human body. This condition causes unbalancing between free radicals and antioxidants.

1. Introduction

Reactive oxygen species (ROS) and free radicals, such as anion superoxide (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are continuous increases in the human body (Phaniendra *et al.*, 2014). The high level of free radicals compared to antioxidants leading to oxidative stress conditions. This condition is involved in some chronic and low-inflammation diseases, such as insulin resistance in type-2 diabetes, rheumatoid arthritis, cardiovascular diseases, and aging disease (Khansari *et al.*, 2009). Therefore, the body needs exogenous antioxidants through functional food products, fruits, vegetables, and food supplements (Bouayed and Bohn, 2010).

An antioxidant is a compound with the ability to reduce the harmfulness of the free radicals (Lobo *et al.*, 2010). According to its source, the antioxidant can be divided into two groups include endogenous (primary) antioxidants, such as superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPx), whereas the second group is exogenous (secondary) antioxidants. This type of antioxidant can get from the diet by eating antioxidant-rich foods or food supplements (Bouayed and Bohn, 2010). Water lettuce is one of the aquatic plants which possess antioxidant properties. This plant also contains some bioactive compounds, such as polyphenols, flavonoids, and saponin (Sudirman *et al.*, 2017a; Sudirman *et al.*, 2017b). According to the previous study, water lettuce (*Pistia stratiotes*) methanol extract showed high antioxidant activity when compared to *n*-hexane and ethyl acetate extracts. This extract is composed of polyphenols and flavonoids (Sudirman *et al.*, 2017a). A previous study reported polyphenols reduced the harmfulness of the free radical by transferring the hydrogen (H) atom from their hydroxyl (OH) groups (Foti, 2007).

However, in the previous study, the authors used a crude extract of the water lettuce. Therefore, in the present study, we tried to separate the methanol crude extract by using thin-layer chromatography (TLC) and column chromatography. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014). A previous study reported that different fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019). According to these conditions, we hypothesized that the antioxidant activity of the water lettuce methanol extract will be increased after the separation process. Therefore, this study aimed to investigate the antioxidant activity of methanol fraction from water lettuce (*Pistia stratiotes*) after separation and confirm their hydroxyl group by Fourier-transform infrared.

2. Materials and methods

2.1 Water lettuce extraction

The fresh form of water lettuce (*Pistia stratiotes*) was collected at swampy waters in Palembang city, South Sumatera, Indonesia, and cleaned to remove unwanted materials. Then, it was reduced the size, dried, and extract by the following previous method (Sudirman *et al.*, 2017b). Briefly, 20 g of dried sample was extracted by using 200 mL of methanol (1:10, v/v) at room temperature for 24 hrs. The solution was filtrated by using a filter paper, solvent removed by using a rotary vacuum evaporator, and dried by using an oven dryer. The sample was kept for future analysis. The percent of extraction yield was calculated from the weight of dried extract divided by the weight of the dried sample and multiplied by 100%.

2.2 Separation process

The thin-layer chromatography (TLC) and column chromatography methods have been widely used to separate fractions from plant extracts. The silica TLC plate and silica gel were purchased from Merck KGaA (Darmstadt, Germany). The TLC method in this research followed the previous study (Kagan and Flythe, 2014). Briefly, the plant extract was dissolved in methanol to have a sample concentration. The TLC plate was cut in a piece (2×10 cm) and leaving a 1 cm border on the upper- and bottom sides of the plate. The sample was loaded by using a microliter syringe on the plate band and allow to dry. The plate with the sample was then developed in the cover chamber which containing mixed solvents or eluent (methanol-ethyl acetate-acetone 1:1:1, v/v/v, for this study). After the developing step, the plate was dried and observed bands under visible or UV light (254 nm and 365 nm) then marked bands with a pencil. The mixed solvent was used to further separation by using silica gel column chromatography. Whereas, the column chromatography was performed by the following previous method (Venkatesh *et al.*, 2017). The mixed solvent was loaded into the packed silica column. The seven different separated fractions (confirmed by TLC) were collected, dried, and kept for future analysis.

2.3. Antioxidant activity assay

The antioxidant activity assay was performed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by the previous method (Molyneux, 2004). The DPPH powder was purchased from Merck KGaA (Darmstadt, Germany). Briefly, each of the dried fractions was dissolved in a methanol solvent to make a serial concentration (50 ppm, 100 ppm, 150 ppm, and 200 ppm). Whereas, Vitamin C (containing ascorbic acid) was used as a positive control. A hundred sixty (160) µL of dried-fraction was added into a well containing 40 µL of 0.76 mM of DPPH (10 mg DPPH in 10 mL of methanol). The solution was incubated at 37°C for 30 min. after incubation time, the

absorbance was immediately measured at 517 nm by using spectrophotometry. The percent of inhibition (%) was calculated from absorbance of blank minus absorbance of the sample divided by absorbance of blank and multiplied by 100%.

2.4. Functional group analysis

The functional group was analyzed by using a Fourier transform-infrared (FT-IR) spectroscopy according to the previous methods (Ouhaddouch *et al.*, 2019). Briefly, the sample was placed into the infrared beam (sample holder), then measured in the spectral range between 500 cm^{-1} and 4000 cm^{-1}. Whereas, KBr-pressed disk was used in the FT-IR spectroscopy (Spectrum One Perkin Elmer, Massachusetts, USA).

3. Results and discussion

This study demonstrated the antioxidant activity of the fractions from the Water lettuce (*Pistia stratiotes*) methanol extract. The previous studies reported that the methanol extract is composed of polyphenols, such as flavonoids and tannin (Sudirman *et al.*, 2017a; Sudirman *et al.*, 2017b). These studies also reported that methanol extract showed the highest antioxidant activity compared to *n*-hexane and ethyl acetate extracts. In addition, methanol extract also shows a high yield extract (Benhammou *et al.*, 2009). Therefore, this present study only separated the methanol extract from water lettuce to some fractions. The yield of water lettuce methanol extract was about 16.16%. A previous study also reported that methanol is the best solvent for the extraction of leaves of *Acalypha wilkesiana* and *Atriplex halimus* were about 14.67% and 24.00%, respectively (Benhammou *et al.*, 2009; Anokwuru *et al.*, 2016). Methanol has been widely used for bioactive extraction from the plant. Methanol is also more efficient in the extraction of polyphenols especially the low molecular weight (MW) of polyphenols (Do *et al.*, 2014).

The fractions from methanol extract were separated by using thin-layer chromatography (TLC) and column chromatography methods. Based on the TLC results, we found that the best-mixed solvent is composed of methanol, ethyl acetate, and acetone with the ratio of 1:1:1, as shown in Figure 1A. According to this result, this eluent was used for the mobile phase in column chromatography. After separation by column chromatography, the result showed that there are seven fractions of the methanol extracts as shown in Figure 1B. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014).

The antioxidant activity of the fraction was evaluated by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The DPPH method was widely used to measure the antioxidant

activity of the extract due to its speed, simplicity, and low cost (Alam *et al.*, 2013). As shown in Table 1, third (F3) and fifth (F5) fractions possessed highly antioxidant activities with the half-maximum inhibitory concentration (IC_{50}) was about 131.66 ppm and 184.62 ppm, respectively. Whereas, other fractions showed weak antioxidant activities with the IC_{50} more than 200 ppm (Molyneux, 2004). A previous study reported that IC_{50} of crude methanol extract of water lettuce was about 147.60 ppm (Sudirman *et al.*, 2017a). The different fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019).

According to the antioxidant activity assay, the highest activity (third, F3) fraction was continued for analysis of its functional group by using Fourier transform-infrared (FT-IR) spectroscopy then compared to the seventh fraction (F7) as a representative from the fraction of weak antioxidant activity as shown in Figure 2 and Table 2. A previous study reported that different bounds of the molecule also show different vibrational frequencies of FTIR spectroscopy, such as C–C, C=C, C–O, C=O, O–H, and N–H bonds (Altemimi *et al.*, 2017). The functional group of each wavenumber in Table 2 was evaluated according to the previous reference (Ouhaddouch *et al.*, 2019). Figure 2 showed that the O-H bond in the F3 fraction (3432.90 cm^{-1}) more than the F7 fraction (3366.06 cm^{-1}). The number of OH groups bound to the aromatic ring is positively correlative with the antioxidant activity of polyphenols (Zielinska-Blizniewska *et al.*, 2019).

4. Conclusion

Overall, the bioactive compound from water lettuce (*Pistia stratiotes*) was successfully extracted by methanol solvent. The methanol extract showed seven fractions after separation by thin-layer chromatography and column chromatography. Whereas, the third fraction possessed high antioxidant activity. The Fourier transform-infrared confirmed that the hydroxyl group of the polyphenols play an important role in their antioxidant activity.

Conflict of interest.

The authors declare no conflict of interest.

Acknowledgments

We are thankful to the Indonesian Ministry of Research, Technology, and Higher Education (2017-2018) to support this study.

References

Alam, M.N., Bristi, N.J. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. and Lightfoot, D. (2017). Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42.

Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O. and Okebugwu, P. (2016). Effect of extraction solvents on phenolic, flavonoid, and antioxidant activities of three Nigerian medicinal plants. *Nature and Science*, 9(7), 53-61.

Benhammou, N., Bekkara, F.A. and Panovska, K.T. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus Chimie*, 12(12), 1259-1266.

Bouayed, J. and Bohn, T. (2010). Exogenous antioxidants—Double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevity*, 3(4), 228-237.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296-302.

Foti, M.C. (2007). Antioxidant properties of phenols. *Journal of Pharmacy and Pharmacology*, 59(12), 1673-1685.

Kagan, I.A. and Flythe, M.D. (2014). Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. *Journal of Visualized Experiments*, 85, e51411.

Khansari, N., Shakiba, Y. and Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation and Allergy Drug Discovery*, 3(1), 73-80.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8), 118.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211-219.

Ouhaddouch, H., Cheikh, A., Idrissi, M.O.B., Draoui, M. and Bouatia, M. (2019). FT-IR spectroscopy applied for identification of a mineral drug substance in drug products: Application to bentonite. *Journal of Spectroscopy* 2019, 1-6.

Phaniendra, A., Jestadi, D.B. and Periyasamy, L. (2014). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30(1), 11-26.

Sudirman, S., Herpandi, H., Lestari, S.D. and Wasahla, W. (2017a). Phytochemicals screening and antioxidant activity of water lettuce (*Pistia Stratiotes*) extract. *European Journal of Scientific Research*, 146(3), 234-238.

Sudirman, S., Herpandi, H., Nopianti, R., Dwita Lestari, S., Wasahla, W. and Mareta, H. (2017b). Phenolic contents, tannin, vitamin C, and vitamin E of water lettuce (*Pistia stratiotes*). *Oriental Journal of Chemistry*, 33(6), 3173-3176.

Triadisti, N., Sauriasari, R. and Elya, B. (2018). Antioxidant activity of fractions from *Garcinia hombroniana* Pierre leaves extracts. *Pharmacognosy Journal*, 10(4), 682-685.

Venkatesh, U., Javarasetty, C. and Murari, S.K. (2017). Purification and fractional analysis of methanolic extract of *Wedelia trilobata* possessing apoptotic and anti-leukemic activity. *African Journal of Traditional, Complementary and Alternative Medicines*, 14(3), 167-174.

Zhao, Q.-C., Zhao, J.-Y., Ahn, D.U., Jin, Y.-G. and Huang, X. (2019). Separation and identification of highly efficient antioxidant peptides from eggshell membrane. *Antioxidants*, 8(10), 495.

Zielinska-Blizniewska, H., Sitarek, P., Merecz-Sadowska, A., Malinowska, K., Zajdel, K., Jablonska, M., Sliwinski, T. and Zajdel, R. (2019). Plant extracts and reactive oxygen species as two counteracting agents with anti- and pro-obesity properties. *International Journal of Molecular Sciences* 20(18), 4556.

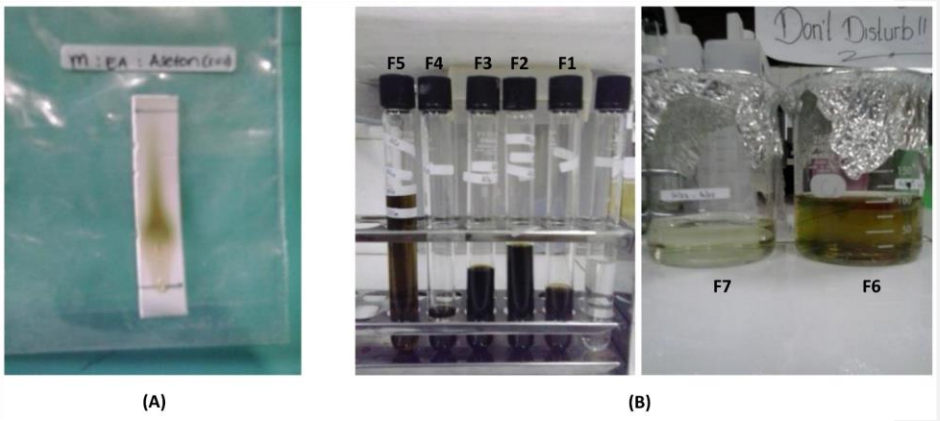


Figure 1. Methanol Extract Separation: **(A)** Thin-Layer Chromatography and **(B)** the Separated-fractions after Separation Process by using Column Chromatography. F1 – F7 are the sample fractions.

Commented [G3]: There are labels of F1 – F7. You should write in the figure note that F1 – F7 are samples.

Commented [SS4R3]: F1 – F7 are the sample fractions.

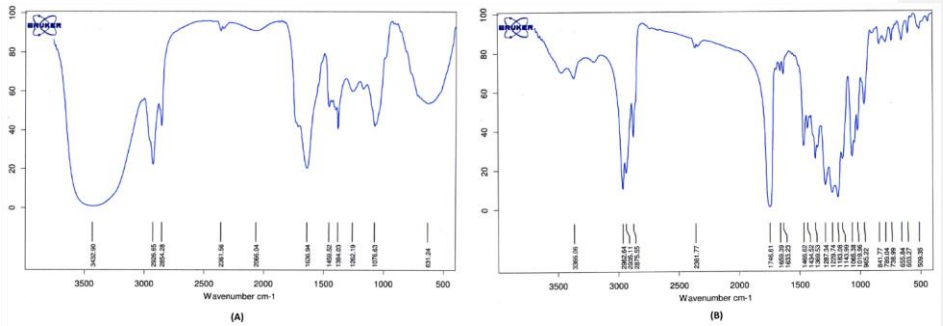


Figure 2. The Fourier Transform-infrared (FT-IR) Spectrums of the **(A)** Third Fraction, F3 and **(B)** Seventh Fraction, F7.

Table 1. The antioxidant activity of the fractions.

Sample	F1	F2	F3	F4	F5	F6	F7	VC
IC_{50} (ppm)	642.97	203.90	131.66	459.61	184.62	>2000	>2000	4.14

Abbreviations: F, sample fraction; IC_{50}, Half-maximum inhibitory concentration; VC, vitamin C as a positive control.

Table 2. The functional groups of third fraction and seventh fraction.

Sample	Wavenumber (cm^{-1})	Wavenumber reference (cm^{-1})	Functional groups
Third fraction (F3)	3432.90	3200-3500	O-H
	2926.65	2800-3000	C-H
	2854.28		
	1636.94	1560-1640	N-H
	1459.52	1400-1500	C-H
	1076.63	1020-1250	C-N, C-O, C-C
Seventh fraction (F7)	3366.06	3200-3500	O-H
	2962.64	2800-3000	C-H
	2935.11		
	2875.55		
	1659.39	1560-1640	N-H
	1633.23		
	1466.62	1400-1500	C-H
	1434.52		
	1065.38	1020-1250	C-N, C-O, C-C
	1019.96		

Manuscript accepted

FR-2020-578 - Decision on your manuscript External Inbox ×

Food Research <foodresearch.my@outlook.com> Mon, 18 Jan 2021, 16:02
to me

Dear Dr Sabri,

It is a pleasure to accept your manuscript for publication in Food Research journal. Please refer to the attachment for your acceptance letter.

Please note that all accepted manuscripts are subjected to Article Processing Charges (APC) as the Journal will provide full publishing services. Please fill in the article processing fee form attached with this letter and revert to us within five (5) working days. Once we have received the form, your article will be transferred to production.

Thank you for your fine contribution. We look forward to your continued contributions to the Journal.

Sincerely,
Dr. Vivian New
Editor
Food Research

Galley proof and invoice

Food Research <foodresearch.my@outlook.com> 25 Apr 2021, 20:00
to me

Dear Dr Sudirman,

Please refer to the attachment for the galley proof of your manuscript FR-2020-578 entitled 'Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract' . Please check the content of the galley proof. If there are any mistakes, please comment and highlight in the PDF itself and revert to us within two (2) days of receipt. Once we have finalized the PDF version, your manuscript will be published online for early viewing.

Please see the attachment for the invoice INV21083. We hope that you can make the payment as soon as possible before 16 May 2021 for us to complete the publication of your manuscript. The manuscript information e.g. volume, issue, page numbers and DOI, will be provided once we have received the payment.

Thanks & Regards,
Vivian New
Editor
Food Research

From: Food Research <foodresearch.my@outlook.com>
Sent: Friday, 23 April, 2021 8:00 PM
To: Sabri Sudirman UNSRI <sabrisudirman@unsri.ac.id>
Subject: Re: FR-2020-578 - Article Production

2 Attachments • Scanned by Gmail

Invoice payment

Sabri Sudirman UNSRI <sabrisudirman@unsri.ac.id> 26 Apr 2021, 14:58
to Food

Dear,
Vivian New
Editor
Food Research

Here, We send the **Payment Proof** of the article **FR-2020-578.**
And we have checked the proof and **have no comment** about the galley proof.
Thank you.

Best regards
Sabri Sudirman
Corresponding author

One attachment • Scanned by Gmail

Food Research <foodresearch.my@outlook.com> 26 Apr 2021, 15:06
to me

Dear Dr Sudirman,

Thank you very much for the payment. I'll notify you on the article publication by this week.

Thanks & Regards,
Vivian New
Editor
Food Research

FOOD
RESEARCH

18th January 2021

Dear Dr Sudirman, S.

ACCEPTANCE LETTER

Food Research, is pleased to inform you that the following manuscript has been accepted for publication in Food Research journal.

Manuscript Title	:	Antioxidant activity of the fractions from water lettuce (Pistia stratiotes) extract
Authors	:	Herpandi, Lestari, S.D., Bastian and Sudirman, S.

We thank you for your fine contribution to the Food Research journal and encourage you to submit other articles to the Journal.

Yours sincerely,

Professor Dr. Son Radu
Chief Editor
Food Research

Food Research (2021) : pp - pp
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

Herpandi, Lestari, S.D., Bastian and *Sudirman, S.

Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya, Ogan Ilir Regency 30862, South Sumatra, Indonesia

Article history:
Received: 9 October 2020
Received in revised form: 14 November 2020
Accepted: 18 January 2021
Available Online:

Keywords:
Antioxidant,
Free radicals,
Hydroxyl group,
Pistia stratiotes,
Separation

DOI:

Abstract

Free radicals including reactive oxygen species are continuously increasing in the human body. This condition causes the unbalance between free radicals and antioxidants in the human body. An antioxidant is a compound with the ability to reduce the harmfulness of the free radical. This study aimed to determine the antioxidant activity of fractions and analyzed the functional groups of water lettuce (*Pistia stratiotes*) methanol extract. The separation process was performed by using thin-layer chromatography (TLC) and column chromatography. The separated fractions were measured for their antioxidant activity by using the 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The functional groups of each fraction were determined by using Fourier-transform infrared (FT-IR) spectroscopy. The separation of water lettuce extract by using column chromatography produced seven fractions with different colors and confirmed by using TLC. The antioxidant activity showed the highest activity in the third fraction with a half-maximal inhibitory concentration (IC_{50}) value of 131.66 ppm. The fifth fraction with the IC_{50} was about 184.62 ppm. Whereas, the first, second, fourth, sixth, and seventh fractions were relatively weak with the IC_{50} more than 200 ppm. The FT-IR spectrum also showed that the intensity hydroxyl group in the third fraction higher than the seventh fraction.

1. Introduction

Reactive oxygen species (ROS) and free radicals, such as anion superoxide (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are continuous increases in the human body (Phaniendra *et al.*, 2014). The high level of free radicals compared to antioxidants leading to oxidative stress conditions. This condition is involved in some chronic and low-inflammation diseases, such as insulin resistance in type-2 diabetes, rheumatoid arthritis, cardiovascular diseases, and aging disease (Khansari *et al.*, 2009). Therefore, the body needs exogenous antioxidants through functional food products, fruits, vegetables, and food supplements (Bouayed and Bohn, 2010).

An antioxidant is a compound with the ability to reduce the harmfulness of the free radicals (Lobo *et al.*, 2010). According to its source, the antioxidant can be divided into two groups include endogenous (primary) antioxidants, such as superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPx), whereas the second group is exogenous (secondary) antioxidants. This type of antioxidant can get from the diet by eating antioxidant-rich foods or food supplements (Bouayed and Bohn, 2010). Water lettuce is one of the aquatic plants which possess antioxidant properties. This plant also contains some bioactive compounds, such as polyphenols, flavonoids, and saponin (Sudirman, Herpandi, Lestari *et al.*, 2017; Sudirman, Herpandi, Nopianti *et al.*, 2017). According to the previous study, water lettuce (*Pistia stratiotes*) methanol extract showed high antioxidant activity when compared to *n*-hexane and ethyl acetate extracts. This extract is composed of polyphenols and flavonoids (Sudirman, Herpandi, Lestari *et al.*, 2017). A previous study reported polyphenols reduced the harmfulness of the free radical by transferring the hydrogen (H) atom from their hydroxyl (OH) groups (Foti, 2007).

However, in the previous study, the authors used a crude extract of the water lettuce. Therefore, in the present study, we tried to separate the methanol crude extract by using thin-layer chromatography (TLC) and column chromatography. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014). A previous study reported that different

*Corresponding author.
Email: *sabrisudirman@unsri.ac.id*

eISSN: 2550-2166

fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019). According to these conditions, we hypothesized that the antioxidant activity of the water lettuce methanol extract will be increased after the separation process. Therefore, this study aimed to investigate the antioxidant activity of methanol fraction from water lettuce (*Pistia stratiotes*) after separation and confirm their hydroxyl group by Fourier-transform infrared.

2. Materials and methods

2.1 Water lettuce extraction

The fresh form of water lettuce (*Pistia stratiotes*) was collected at swampy waters in Palembang city, South Sumatera, Indonesia, and cleaned to remove unwanted materials. Then, it was reduced the size, dried, and extract by the following previous method (Sudirman, Herpandi, Nopianti *et al.*, 2017). Briefly, 20 g of dried sample was extracted by using 200 mL of methanol (1:10, v/v) at room temperature for 24 hrs. The solution was filtrated by using a filter paper, solvent removed by using a rotary vacuum evaporator, and dried by using an oven dryer. The sample was kept for future analysis. The percent of extraction yield was calculated from the weight of dried extract divided by the weight of the dried sample and multiplied by 100%.

2.2 Separation process

The thin-layer chromatography (TLC) and column chromatography methods have been widely used to separate fractions from plant extracts. The silica TLC plate and silica gel were purchased from Merck KGaA (Darmstadt, Germany). The TLC method in this research followed the previous study (Kagan and Flythe, 2014). Briefly, the plant extract was dissolved in methanol to have a sample concentration. The TLC plate was cut in a piece (2×10 cm) and leaving a 1 cm border on the upper- and bottom sides of the plate. The sample was loaded by using a microliter syringe on the plate band and allow to dry. The plate with the sample was then developed in the cover chamber which containing mixed solvents or eluent (methanol-ethyl acetate-acetone 1:1:1, v/v/v, for this study). After the developing step, the plate was dried and observed bands under visible or UV light (254 nm and 365 nm) then marked bands with a pencil. The mixed solvent was used to further separation by using silica gel column chromatography. Whereas, the column chromatography was performed by the following previous method (Venkatesh *et al.*, 2017). The mixed solvent was loaded into the packed silica column. The seven different separated fractions (confirmed by TLC) were collected, dried, and kept for future analysis.

2.3. Antioxidant activity assay

The antioxidant activity assay was performed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by the previous method (Molyneux, 2004). The DPPH powder was purchased from Merck KGaA (Darmstadt, Germany). Briefly, each of the dried fractions was dissolved in a methanol solvent to make a serial concentration (50 ppm, 100 ppm, 150 ppm, and 200 ppm). Whereas, Vitamin C (containing ascorbic acid) was used as a positive control. A hundred sixty (160) µL of dried-fraction was added into a well containing 40 µL of 0.76 mM of DPPH (10 mg DPPH in 10 mL of methanol). The solution was incubated at 37°C for 30 mins. after incubation time, the absorbance was immediately measured at 517 nm by using spectrophotometry. The percent of inhibition (%) was calculated from absorbance of blank minus absorbance of the sample divided by absorbance of blank and multiplied by 100%.

2.4. Functional group analysis

The functional group was analyzed by using a Fourier transform-infrared (FT-IR) spectroscopy according to the previous methods (Ouhaddouch *et al.*, 2019). Briefly, the sample was placed into the infrared beam (sample holder), then measured in the spectral range between 500 cm^{-1} and 4000 cm^{-1}. Whereas, KBr-pressed disk was used in the FT-IR spectroscopy (Spectrum One Perkin Elmer, Massachusetts, USA).

3. Results and discussion

This study demonstrated the antioxidant activity of the fractions from the Water lettuce (*Pistia stratiotes*) methanol extract. The previous studies reported that the methanol extract is composed of polyphenols, such as flavonoids and tannin (Sudirman, Herpandi, Lestari *et al.*, 2017; Sudirman, Herpandi, Nopianti *et al.*, 2017). These studies also reported that methanol extract showed the highest antioxidant activity compared to *n*-hexane and ethyl acetate extracts. In addition, methanol extract also shows a high yield extract (Benhammou *et al.*, 2009). Therefore, this present study only separated the methanol extract from water lettuce to some fractions. The yield of water lettuce methanol extract was about 16.16%. A previous study also reported that methanol is the best solvent for the extraction of leaves of *Acalypha wilkesiana* and *Atriplex halimus* were about 14.67% and 24.00%, respectively (Benhammou *et al.*, 2009; Anokwuru *et al.*, 2016). Methanol has been widely used for bioactive extraction from the plant. Methanol is also

more efficient in the extraction of polyphenols especially the low molecular weight (MW) of polyphenols (Do *et al.*, 2014).

The fractions from methanol extract were separated by using thin-layer chromatography (TLC) and column chromatography methods. Based on the TLC results, we found that the best-mixed solvent is composed of methanol, ethyl acetate, and acetone with the ratio of 1:1:1, as shown in Figure 1A. According to this result, this eluent was used for the mobile phase in column chromatography. After separation by column chromatography, the result showed that there are seven fractions of the methanol extracts as shown in Figure 1B. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014).

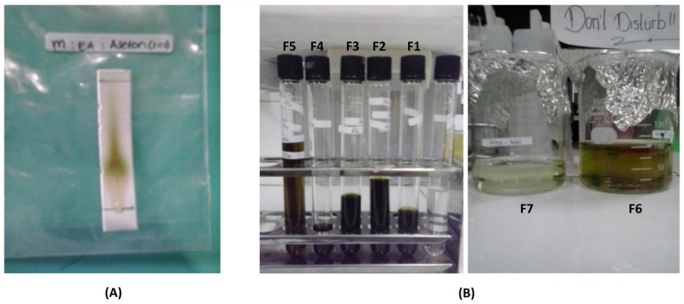


Figure 1. Methanol Extract Separation: **(A)** Thin-Layer Chromatography and **(B)** the Separated-fractions after Separation Process by using Column Chromatography. F1 – F7 are the sample fractions.

The antioxidant activity of the fraction was evaluated by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The DPPH method was widely used to measure the antioxidant activity of the extract due to its speed, simplicity, and low cost (Alam *et al.*, 2013). As shown in Table 1, third (F3) and fifth (F5) fractions possessed highly antioxidant activities with the half-maximum inhibitory concentration (IC_{50}) was about 131.66 ppm and 184.62 ppm, respectively. Whereas, other fractions showed weak antioxidant activities with the IC_{50} more than 200 ppm (Molyneux, 2004). A previous study reported that IC_{50} of crude methanol extract of water lettuce was about 147.60 ppm (Sudirman, Herpandi, Lestari*et al.*, 2017). The different fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019).

According to the antioxidant activity assay, the highest activity (third, F3) fraction was continued for analysis of its functional group by using Fourier transform-infrared (FT-IR) spectroscopy then compared to the seventh fraction (F7) as a representative from the fraction of weak antioxidant activity as shown in Figure 2 and Table 2. A previous study reported that different bounds of the molecule also show different vibrational frequencies of FTIR spectroscopy, such as C–C, C=C, C –O, C=O, O–H, and N–H bonds (Altemimi *et al.*, 2017). The functional group of each wavenumber in Table 2 was evaluated according to the previous reference (Ouhaddouch *et al.*, 2019). Figure 2 showed that the O-H bond in the F3 fraction (3432.90 cm^{-1}) more than the F7 fraction (3366.06 cm^{-1}). The number of OH groups bound to the aromatic ring is positively correlative with

Table 1. The antioxidant activity of the fractions.

Sample	F1	F2	F3	F4	F5	F6	F7	VC
IC_{50} (ppm)	642.97	203.9	131.66	459.61	184.62	>2000	>2000	4.14

F: sample fraction, IC_{50}: Half-maximum inhibitory concentration, VC: vitamin C as a positive control.

Table 2. The functional groups of third fraction and seventh fraction.

Sample	Wavenumber (cm^{-1})	Wavenumber reference (cm^{-1})	Functional groups
Third fraction (F3)	3432.9	3200-3500	O-H
	2926.65 2854.28	2800-3000	C-H
	1636.94	1560-1640	N-H
	1459.52	1400-1500	C-H
	1076.63	1020-1250	C-N, C-O, C-C
Seventh fraction (F7)	3366.06	3200-3500	O-H
	2962.64 2935.11 2875.55	2800-3000	C-H
	1659.39 1633.23	1560-1640	N-H
	1466.62 1434.52	1400-1500	C-H
	1065.38 1019.96	1020-1250	C-N, C-O, C-C

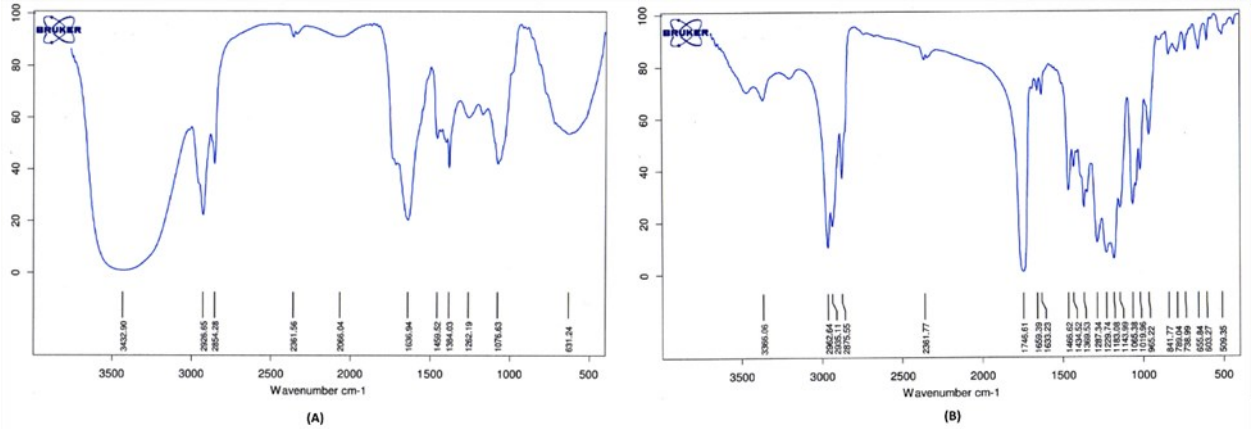


Figure 2. The Fourier Transform-infrared (FT-IR) Spectrums of the (A) Third Fraction, F3 and (B) Seventh Fraction, F7.

the antioxidant activity of polyphenols (Zielinska-Blizniewska *et al.*, 2019).

4. Conclusion

Overall, the bioactive compound from water lettuce (*Pistia stratiotes*) was successfully extracted by methanol solvent. The methanol extract showed seven fractions after separation by thin-layer chromatography and column chromatography. Whereas, the third fraction possessed high antioxidant activity. The Fourier transform-infrared confirmed that the hydroxyl group of the polyphenols play an important role in their antioxidant activity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We are thankful to the Indonesian Ministry of Research, Technology, and Higher Education (2017-2018) to support this study.

References

Alam, M.N., Bristi, N.J. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152. https://doi.org/10.1016/j.jsps.2012.05.002

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. and Lightfoot, D. (2017). Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42. https://doi.org/10.3390/plants6040042

Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O. and Okebugwu, P. (2016). Effect of extraction solvents on phenolic, flavonoid, and antioxidant activities of three Nigerian medicinal plants. *Nature and Science*, 9(7), 53-61.

Benhammou, N., Bekkara, F.A. and Panovska, K.T. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus Chimie*, 12(12), 1259-1266. https://doi.org/10.1016/j.crci.2009.02.004

Bouayed, J. and Bohn, T. (2010). Exogenous antioxidants—Double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevity*, 3(4), 228-237. https://doi.org/10.4161/oxim.3.4.12858

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296-302. https://doi.org/10.1016/j.jfda.2013.11.001

Foti, M.C. (2007). Antioxidant properties of phenols. *Journal of Pharmacy and Pharmacology*, 59(12), 1673-1685. https://doi.org/10.1211/jpp.59.12.0010

Kagan, I.A. and Flythe, M.D. (2014). Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. *Journal of Visualized Experiments*, 85, e51411. https://doi.org/10.3791/51411

Khansari, N., Shakiba, Y. and Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation and Allergy Drug Discovery*, 3(1), 73-80. https://doi.org/10.2174/187221309787158371

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4 (8), 118. https://doi.org/10.4103/0973-7847.70902

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211-219.

Ouhaddouch, H., Cheikh, A., Idrissi, M.O.B., Draoui, M. and Bouatia, M. (2019). FT-IR spectroscopy applied for identification of a mineral drug substance in drug products: Application to bentonite. *Journal of Spectroscopy*, 2019, 2960845. https://doi.org/10.1155/2019/2960845

Phaniendra, A., Jestadi, D.B. and Periyasamy, L. (2014). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30(1), 11-26. https://doi.org/10.1007/s12291-014-0446-0

Sudirman, S., Herpandi, H., Lestari, S.D. and Wasahla, W. (2017). Phytochemicals screening and antioxidant activity of water lettuce (*Pistia Stratiotes*) extract. *European Journal of Scientific Research*, 146(3), 234-238.

Sudirman, S., Herpandi, H., Nopianti, R., Dwita Lestari, S., Wasahla, W. and Mareta, H. (2017). Phenolic contents, tannin, vitamin C, and vitamin E of water lettuce (*Pistia stratiotes*). *Oriental Journal of Chemistry*, 33(6), 3173-3176. https://doi.org/10.13005/ojc/330661

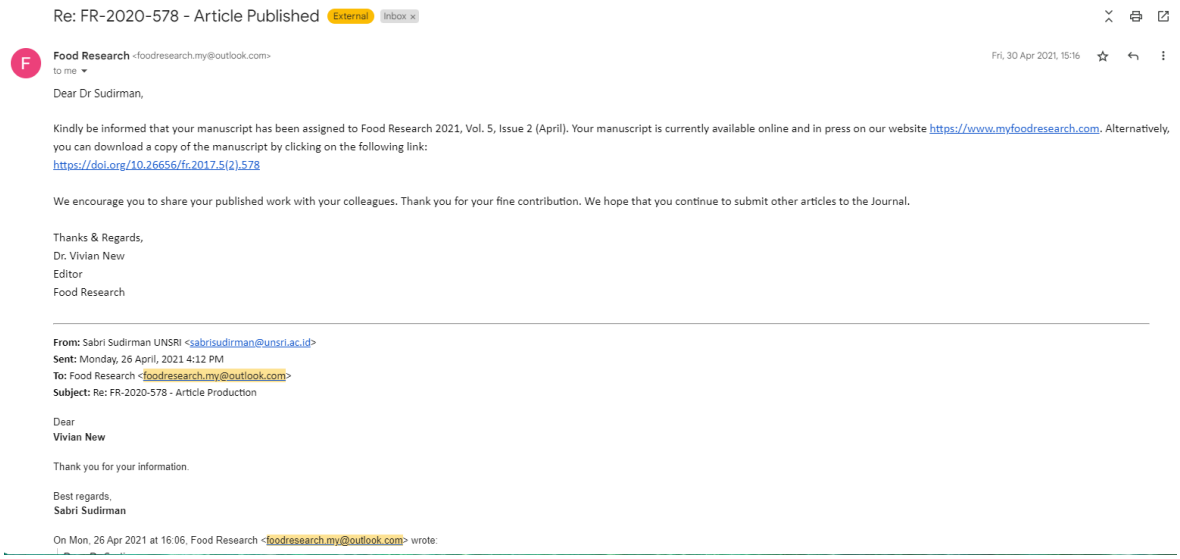
Triadisti, N., Sauriasari, R. and Elya, B. (2018). Antioxidant activity of fractions from *Garcinia hombroniana* Pierre leaves extracts. *Pharmacognosy Journal*, 10(4), 682-685. https://doi.org/10.5530/pj.2018.4.112

Venkatesh, U., Javarasetty, C. and Murari, S.K. (2017). Purification and fractional analysis of methanolic extract of *Wedelia trilobata* possessing apoptotic and anti-leukemic activity. *African Journal of Traditional, Complementary and Alternative Medicines*, 14(3), 167-174. https://doi.org/10.21010/ajtcam.v14i3.18

Zhao, Q.-C., Zhao, J.-Y., Ahn, D.U., Jin, Y.-G. and Huang, X. (2019). Separation and identification of highly efficient antioxidant peptides from eggshell membrane. *Antioxidants*, 8(10), 495. https://doi.org/10.3390/antiox8100495

Zielinska-Blizniewska, H., Sitarek, P., Merecz-Sadowska, A., Malinowska, K., Zajdel, K., Jablonska, M., Sliwinski, T. and Zajdel, R. (2019). Plant extracts and reactive oxygen species as two counteracting agents with anti- and pro-obesity properties. *International Journal of Molecular Sciences* 20(18), 4556. https://doi.org/10.3390/ijms20184556

Article published



Re: FR-2020-578 - Article Published External Inbox ×

Food Research <foodresearch.my@outlook.com> Fri, 30 Apr 2021, 15:16
to me

Dear Dr Sudirman,

Kindly be informed that your manuscript has been assigned to Food Research 2021, Vol. 5, Issue 2 (April). Your manuscript is currently available online and in press on our website https://www.myfoodresearch.com. Alternatively, you can download a copy of the manuscript by clicking on the following link:
https://doi.org/10.26656/fr.2017.5(2).578

We encourage you to share your published work with your colleagues. Thank you for your fine contribution. We hope that you continue to submit other articles to the Journal.

Thanks & Regards,
Dr. Vivian New
Editor
Food Research

From: Sabri Sudirman UNSRI <sabrisudirman@unsri.ac.id>
Sent: Monday, 26 April, 2021 4:12 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2020-578 - Article Production

Dear
Vivian New

Thank you for your information.

Best regards,
Sabri Sudirman

On Mon, 26 Apr 2021 at 16:06, Food Research <foodresearch.my@outlook.com> wrote:

Food Research 5 (2) : 451 - 455 (April 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

Herpandi, Lestari, S.D., Bastian and *Sudirman, S.

Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya, Ogan Ilir Regency 30862, South Sumatra, Indonesia

Article history:
Received: 9 October 2020
Received in revised form: 14 November 2020
Accepted: 18 January 2021
Available Online: 29 April 2021

Keywords:
Antioxidant,
Free radicals,
Hydroxyl group,
Pistia stratiotes,
Separation

DOI:
https://doi.org/10.26656/fr.2017.5(2).578

Abstract

Free radicals including reactive oxygen species are continuously increasing in the human body. This condition causes the unbalance between free radicals and antioxidants in the human body. An antioxidant is a compound with the ability to reduce the harmfulness of the free radical. This study aimed to determine the antioxidant activity of fractions and analyzed the functional groups of water lettuce (*Pistia stratiotes*) methanol extract. The separation process was performed by using thin-layer chromatography (TLC) and column chromatography. The separated fractions were measured for their antioxidant activity by using the 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The functional groups of each fraction were determined by using Fourier-transform infrared (FT-IR) spectroscopy. The separation of water lettuce extract by using column chromatography produced seven fractions with different colors and confirmed by using TLC. The antioxidant activity showed the highest activity in the third fraction with a half-maximal inhibitory concentration (IC_{50}) value of 131.66 ppm. The fifth fraction with the IC_{50} was about 184.62 ppm. Whereas, the first, second, fourth, sixth, and seventh fractions were relatively weak with the IC_{50} more than 200 ppm. The FT-IR spectrum also showed that the intensity hydroxyl group in the third fraction higher than the seventh fraction.

1. Introduction

Reactive oxygen species (ROS) and free radicals, such as anion superoxide (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are continuous increases in the human body (Phaniendra *et al.*, 2014). The high level of free radicals compared to antioxidants leading to oxidative stress conditions. This condition is involved in some chronic and low-inflammation diseases, such as insulin resistance in type-2 diabetes, rheumatoid arthritis, cardiovascular diseases, and aging disease (Khansari *et al.*, 2009). Therefore, the body needs exogenous antioxidants through functional food products, fruits, vegetables, and food supplements (Bouayed and Bohn, 2010).

An antioxidant is a compound with the ability to reduce the harmfulness of the free radicals (Lobo *et al.*, 2010). According to its source, the antioxidant can be divided into two groups include endogenous (primary) antioxidants, such as superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPx), whereas the second group is exogenous (secondary) antioxidants. This type of antioxidant can get from the diet by eating antioxidant-rich foods or food supplements (Bouayed and Bohn, 2010). Water lettuce is one of the aquatic plants which possess antioxidant properties. This plant also contains some bioactive compounds, such as polyphenols, flavonoids, and saponin (Sudirman, Herpandi, Lestari *et al.*, 2017; Sudirman, Herpandi, Nopianti *et al.*, 2017). According to the previous study, water lettuce (*Pistia stratiotes*) methanol extract showed high antioxidant activity when compared to *n*-hexane and ethyl acetate extracts. This extract is composed of polyphenols and flavonoids (Sudirman, Herpandi, Lestari *et al.*, 2017). A previous study reported polyphenols reduced the harmfulness of the free radical by transferring the hydrogen (H) atom from their hydroxyl (OH) groups (Foti, 2007).

However, in the previous study, the authors used a crude extract of the water lettuce. Therefore, in the present study, we tried to separate the methanol crude extract by using thin-layer chromatography (TLC) and column chromatography. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014). A previous study reported that different

*Corresponding author.
Email: *sabrisudirman@unsri.ac.id*

eISSN: 2550-2166

fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019). According to these conditions, we hypothesized that the antioxidant activity of the water lettuce methanol extract will be increased after the separation process. Therefore, this study aimed to investigate the antioxidant activity of methanol fraction from water lettuce (*Pistia stratiotes*) after separation and confirm their hydroxyl group by Fourier-transform infrared.

2. Materials and methods

2.1 Water lettuce extraction

The fresh form of water lettuce (*Pistia stratiotes*) was collected at swampy waters in Palembang city, South Sumatera, Indonesia, and cleaned to remove unwanted materials. Then, it was reduced the size, dried, and extract by the following previous method (Sudirman, Herpandi, Nopianti *et al.*, 2017). Briefly, 20 g of dried sample was extracted by using 200 mL of methanol (1:10, v/v) at room temperature for 24 hrs. The solution was filtrated by using a filter paper, solvent removed by using a rotary vacuum evaporator, and dried by using an oven dryer. The sample was kept for future analysis. The percent of extraction yield was calculated from the weight of dried extract divided by the weight of the dried sample and multiplied by 100%.

2.2 Separation process

The thin-layer chromatography (TLC) and column chromatography methods have been widely used to separate fractions from plant extracts. The silica TLC plate and silica gel were purchased from Merck KGaA (Darmstadt, Germany). The TLC method in this research followed the previous study (Kagan and Flythe, 2014). Briefly, the plant extract was dissolved in methanol to have a sample concentration. The TLC plate was cut in a piece (2×10 cm) and leaving a 1 cm border on the upper- and bottom sides of the plate. The sample was loaded by using a microliter syringe on the plate band and allow to dry. The plate with the sample was then developed in the cover chamber which containing mixed solvents or eluent (methanol-ethyl acetate-acetone 1:1:1, v/v/v, for this study). After the developing step, the plate was dried and observed bands under visible or UV light (254 nm and 365 nm) then marked bands with a pencil. The mixed solvent was used to further separation by using silica gel column chromatography. Whereas, the column chromatography was performed by the following previous method (Venkatesh *et al.*, 2017). The mixed solvent was loaded into the packed silica column. The seven different separated fractions (confirmed by TLC) were collected, dried, and kept for future analysis.

2.3. Antioxidant activity assay

The antioxidant activity assay was performed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by the previous method (Molyneux, 2004). The DPPH powder was purchased from Merck KGaA (Darmstadt, Germany). Briefly, each of the dried fractions was dissolved in a methanol solvent to make a serial concentration (50 ppm, 100 ppm, 150 ppm, and 200 ppm). Whereas, Vitamin C (containing ascorbic acid) was used as a positive control. A hundred sixty (160) µL of dried-fraction was added into a well containing 40 µL of 0.76 mM of DPPH (10 mg DPPH in 10 mL of methanol). The solution was incubated at 37°C for 30 mins. after incubation time, the absorbance was immediately measured at 517 nm by using spectrophotometry. The percent of inhibition (%) was calculated from absorbance of blank minus absorbance of the sample divided by absorbance of blank and multiplied by 100%.

2.4. Functional group analysis

The functional group was analyzed by using a Fourier transform-infrared (FT-IR) spectroscopy according to the previous methods (Ouhaddouch *et al.*, 2019). Briefly, the sample was placed into the infrared beam (sample holder), then measured in the spectral range between 500 cm^{-1} and 4000 cm^{-1}. Whereas, KBr-pressed disk was used in the FT-IR spectroscopy (Spectrum One Perkin Elmer, Massachusetts, USA).

3. Results and discussion

This study demonstrated the antioxidant activity of the fractions from the Water lettuce (*Pistia stratiotes*) methanol extract. The previous studies reported that the methanol extract is composed of polyphenols, such as flavonoids and tannin (Sudirman, Herpandi, Lestari *et al.*, 2017; Sudirman, Herpandi, Nopianti *et al.*, 2017). These studies also reported that methanol extract showed the highest antioxidant activity compared to *n*-hexane and ethyl acetate extracts. In addition, methanol extract also shows a high yield extract (Benhammou *et al.*, 2009). Therefore, this present study only separated the methanol extract from water lettuce to some fractions. The yield of water lettuce methanol extract was about 16.16%. A previous study also reported that methanol is the best solvent for the extraction of leaves of *Acalypha wilkesiana* and *Atriplex halimus* were about 14.67% and 24.00%, respectively (Benhammou *et al.*, 2009; Anokwuru *et al.*, 2016). Methanol has been widely used for bioactive extraction from the plant. Methanol is also

more efficient in the extraction of polyphenols especially the low molecular weight (MW) of polyphenols (Do *et al.*, 2014).

The fractions from methanol extract were separated by using thin-layer chromatography (TLC) and column chromatography methods. Based on the TLC results, we found that the best-mixed solvent is composed of methanol, ethyl acetate, and acetone with the ratio of 1:1:1, as shown in Figure 1A. According to this result, this eluent was used for the mobile phase in column chromatography. After separation by column chromatography, the result showed that there are seven fractions of the methanol extracts as shown in Figure 1B. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014).

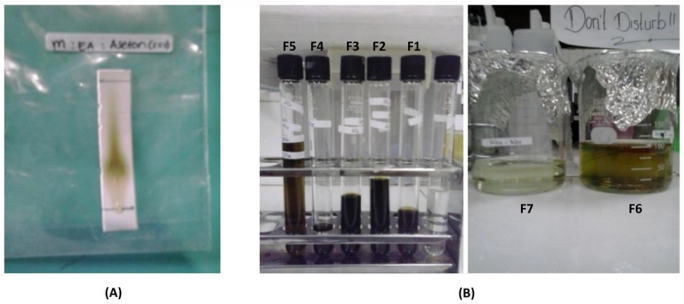


Figure 1. Methanol Extract Separation: **(A)** Thin-Layer Chromatography and **(B)** the Separated-fractions after Separation Process by using Column Chromatography. F1 – F7 are the sample fractions.

The antioxidant activity of the fraction was evaluated by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The DPPH method was widely used to measure the antioxidant activity of the extract due to its speed, simplicity, and low cost (Alam *et al.*, 2013). As shown in Table 1, third (F3) and fifth (F5) fractions possessed highly antioxidant activities with the half-maximum inhibitory concentration (IC_{50}) was about 131.66 ppm and 184.62 ppm, respectively. Whereas, other fractions showed weak antioxidant activities with the IC_{50} more than 200 ppm (Molyneux, 2004). A previous study reported that IC_{50} of crude methanol extract of water lettuce was about 147.60 ppm (Sudirman, Herpandi, Lestari*et al.*, 2017). The different fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019).

According to the antioxidant activity assay, the highest activity (third, F3) fraction was continued for analysis of its functional group by using Fourier transform-infrared (FT-IR) spectroscopy then compared to the seventh fraction (F7) as a representative from the fraction of weak antioxidant activity as shown in Figure 2 and Table 2. A previous study reported that different bounds of the molecule also show different vibrational frequencies of FTIR spectroscopy, such as C–C, C=C, C –O, C=O, O–H, and N–H bonds (Altemimi *et al.*, 2017). The functional group of each wavenumber in Table 2 was evaluated according to the previous reference (Ouhaddouch *et al.*, 2019). Figure 2 showed that the O-H bond in the F3 fraction (3432.90 cm^{-1}) more than the F7 fraction (3366.06 cm^{-1}). The number of OH groups bound to the aromatic ring is positively correlative with

Table 1. The antioxidant activity of the fractions.

Sample	F1	F2	F3	F4	F5	F6	F7	VC
IC_{50} (ppm)	642.97	203.9	131.66	459.61	184.62	>2000	>2000	4.14

F: sample fraction, IC_{50}: Half-maximum inhibitory concentration, VC: vitamin C as a positive control.

Table 2. The functional groups of third fraction and seventh fraction.

Sample	Wavenumber (cm^{-1})	Wavenumber reference (cm^{-1})	Functional groups
Third fraction (F3)	3432.9	3200-3500	O-H
	2926.65 2854.28	2800-3000	C-H
	1636.94	1560-1640	N-H
	1459.52	1400-1500	C-H
	1076.63	1020-1250	C-N, C-O, C-C
Seventh fraction (F7)	3366.06	3200-3500	O-H
	2962.64 2935.11 2875.55	2800-3000	C-H
	1659.39 1633.23	1560-1640	N-H
	1466.62 1434.52	1400-1500	C-H
	1065.38 1019.96	1020-1250	C-N, C-O, C-C

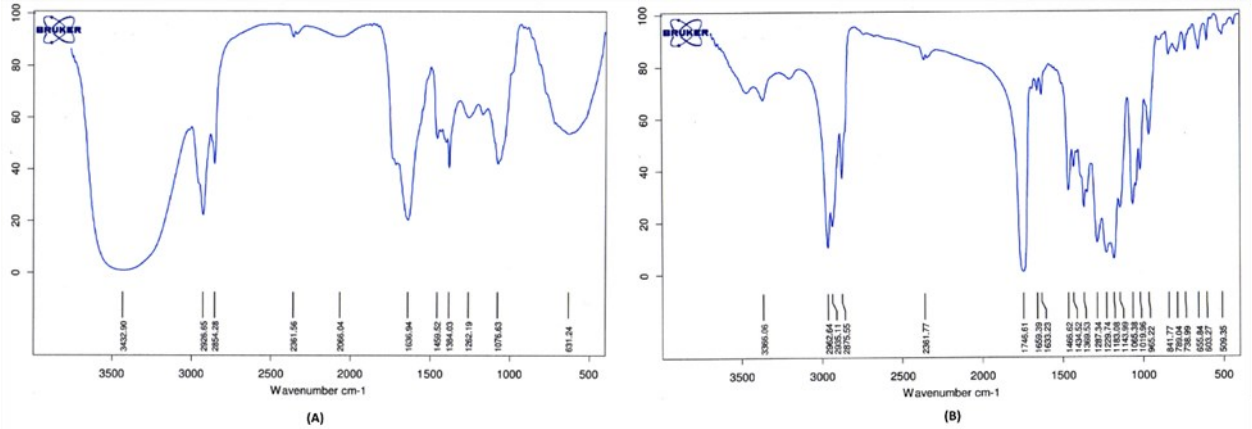


Figure 2. The Fourier Transform-infrared (FT-IR) Spectrums of the (A) Third Fraction, F3 and (B) Seventh Fraction, F7.

the antioxidant activity of polyphenols (Zielinska-Blizniewska *et al.*, 2019).

4. Conclusion

Overall, the bioactive compound from water lettuce (*Pistia stratiotes*) was successfully extracted by methanol solvent. The methanol extract showed seven fractions after separation by thin-layer chromatography and column chromatography. Whereas, the third fraction possessed high antioxidant activity. The Fourier transform-infrared confirmed that the hydroxyl group of the polyphenols play an important role in their antioxidant activity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We are thankful to the Indonesian Ministry of Research, Technology, and Higher Education (2017-2018) to support this study.

References

Alam, M.N., Bristi, N.J. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152. https://doi.org/10.1016/j.jsps.2012.05.002

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. and Lightfoot, D. (2017). Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42. https://doi.org/10.3390/plants6040042

Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O. and Okebugwu, P. (2016). Effect of extraction solvents on phenolic, flavonoid, and antioxidant activities of three Nigerian medicinal plants. *Nature and Science*, 9(7), 53-61.

Benhammou, N., Bekkara, F.A. and Panovska, K.T. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus Chimie*, 12(12), 1259-1266. https://doi.org/10.1016/j.crci.2009.02.004

Bouayed, J. and Bohn, T. (2010). Exogenous antioxidants—Double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevity*, 3(4), 228-237. https://doi.org/10.4161/oxim.3.4.12858

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296-302. https://doi.org/10.1016/j.jfda.2013.11.001

Foti, M.C. (2007). Antioxidant properties of phenols. *Journal of Pharmacy and Pharmacology*, 59(12), 1673-1685. https://doi.org/10.1211/jpp.59.12.0010

Kagan, I.A. and Flythe, M.D. (2014). Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. *Journal of Visualized Experiments*, 85, e51411. https://doi.org/10.3791/51411

Khansari, N., Shakiba, Y. and Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation and Allergy Drug Discovery*, 3(1), 73-80. https://doi.org/10.2174/187221309787158371

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4 (8), 118. https://doi.org/10.4103/0973-7847.70902

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211-219.

Ouhaddouch, H., Cheikh, A., Idrissi, M.O.B., Draoui, M. and Bouatia, M. (2019). FT-IR spectroscopy applied for identification of a mineral drug substance in drug products: Application to bentonite. *Journal of Spectroscopy*, 2019, 2960845. https://doi.org/10.1155/2019/2960845

Phaniendra, A., Jestadi, D.B. and Periyasamy, L. (2014). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30(1), 11-26. https://doi.org/10.1007/s12291-014-0446-0

Sudirman, S., Herpandi, H., Lestari, S.D. and Wasahla, W. (2017). Phytochemicals screening and antioxidant activity of water lettuce (*Pistia Stratiotes*) extract. *European Journal of Scientific Research*, 146(3), 234-238.

Sudirman, S., Herpandi, H., Nopianti, R., Dwita Lestari, S., Wasahla, W. and Mareta, H. (2017). Phenolic contents, tannin, vitamin C, and vitamin E of water lettuce (*Pistia stratiotes*). *Oriental Journal of Chemistry*, 33(6), 3173-3176. https://doi.org/10.13005/ojc/330661

Triadisti, N., Sauriasari, R. and Elya, B. (2018). Antioxidant activity of fractions from *Garcinia hombroniana* Pierre leaves extracts. *Pharmacognosy Journal*, 10(4), 682-685. https://doi.org/10.5530/pj.2018.4.112

Venkatesh, U., Javarasetty, C. and Murari, S.K. (2017). Purification and fractional analysis of methanolic extract of *Wedelia trilobata* possessing apoptotic and anti-leukemic activity. *African Journal of Traditional, Complementary and Alternative Medicines*, 14(3), 167-174. https://doi.org/10.21010/ajtcam.v14i3.18

Zhao, Q.-C., Zhao, J.-Y., Ahn, D.U., Jin, Y.-G. and Huang, X. (2019). Separation and identification of highly efficient antioxidant peptides from eggshell membrane. *Antioxidants*, 8(10), 495. https://doi.org/10.3390/antiox8100495

Zielinska-Blizniewska, H., Sitarek, P., Merecz-Sadowska, A., Malinowska, K., Zajdel, K., Jablonska, M., Sliwinski, T. and Zajdel, R. (2019). Plant extracts and reactive oxygen species as two counteracting agents with anti- and pro-obesity properties. *International Journal of Molecular Sciences* 20(18), 4556. https://doi.org/10.3390/ijms20184556

