8. A green chemistry approach

by Amin Fatoni

Submission date: 02-Feb-2023 02:33PM (UTC+0700)
Submission ID: 2004665548
File name: ch_using_Alternanthera_brasiliana_extract_for_urea_biosensor.pdf (564.16K)
Word count: 94
Character count: 18123

A green chemistry approach using *Alternanthera brasiliana* extract for urea biosensor

Cite as: AIP Conference Proceedings **2553**, 020020 (2022); https://doi.org/10.1063/5.0103678
Published Online: 22 November 2022

Amin Fatoni, Greda Rido Gusti, Zusfahair, et al.

A Green Chemistry Approach using *Alternanthera brasiliana* Extract for Urea Biosensor

Amin Fatoni[1, a)], Greda Rido Gusti[1, b)], Zusfahair[1, c)], Mekar Dwi Anggraeni[2, d)] and Saluma Samanman[3, e)]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jenderal Soedirman, Jl Dr Soeparno, Purwokerto, Indonesia*
[2] *Department of Nursing, Faculty of Health Sciences, Universitas Jenderal Soedirman, Jl Dr Soeparno, Purwokerto, Indonesia*
[3]*Faculty of Science and Technology, Princess of Naradhiwas University, Narathiwat, Thailand*

[a)] Corresponding author: aminfatoni@unsoed.ac.id
[b)] greda@gmail.com
[c)] zusfahair@gmail.com
[d)] mekar.anggraeni@unsoed.ac.id
[e)] saluma.s@pnu.ac.th

Abstract. A biosensor is an analytical method that uses an active biological component that is integrated with electronic equipment to determine the analyte concentration. One of the biosensor methods widely developed is colorimetric-based detection. The purpose of this research was to study the potential of the use of purple spinach (*Alternanthera brasiliana*) leaf extract as a urea biosensor indicator. The research was performed including the leaf extraction, characterization, and application as a urea biosensor indicator. The results showed that the optimum purple spinach leaf extraction using Ethanol-HCl 85:15 (v/v) with an absorbance of 0.802 at a maximum wavelength of 531 nm. Furthermore, the leaf extract showed high stability in acid, low temperature (<4°C) storage, and the addition of reducing agent. The purple spinach leaf as an indicator of urea biosensor showed a linear correlation between the color change and urea concentration, with the regression line of y = -0.0007x + 0.6397 and R2 of 0.961.

INTRODUCTION

Urea is a chemical compound that can be formed biologically in the body of living things, both humans, animals, and plants. In the human body, urea formation occurs as the end product of the nitrogen cycle in the liver. This compound is used in the formation of amino acids as protein elements that are very useful for the body [1]. High levels of urea can cause several diseases such as kidney and liver problems. It is important to routinely determine the urea levels to examine the functions of these organs. One of the most common methods used for urea determination is the spectrophotometric method [2], using diacetyl monoxime to produce a yellow solution and measure the absorbance. Other methods to determine the urea levels are the enzymatic method using urease [3] and the potentiometric method with ion-selective electrode (ISE) [4]. The limitation of the spectrophotometric method is the use of chemical reagents, while the ESI method limitation is non-selective for urea only. One of the developments of selective methods is the biosensor, involving the use of biological sensing elements. A biosensor is an analytical method that uses active biological components that are integrated with electronic equipment to determine the analyte concentration. The biosensors are very interesting to develop because of their selectivity, accuracy, reliability and even have considerable economic prospects [5, 6]. Biosensors also can determine very low concentrations of analytes, in parts *per million* (ppm), *parts per billion* (ppb), and *parts per trillion* (ppt).

Proceedings of the 16th Joint Conference on Chemistry (JCC 2021)
AIP Conf. Proc. 2553, 020020-1–020020-7; https://doi.org/10.1063/5.0103678
Published by AIP Publishing. 978-0-7354-4281-8/$30.00

The development of urea detectors promises the need of new urease determination of easy and inexpensive analysis. The urea biosensor could be applied in health sciences agriculture [7] and hydroponics, such as the determination of urea in soil and surface. A common urea biosensor uses the urease [3, 8, 9] enzyme to convert the urea into ammonium ion. The ammonium ions could be measured based on the changes in pH, resulting in the color change of the pH indicator. Several natural extracts as indicators have been reported such as purple cabbage (*Brassica oleracea* L.), *Etlingera elatior* [10], pineapple shells (*rhoeo discolor*), and *Caesalpinia sappan* [9]. The use of natural indicators in the analytical methods would reduce the hazardous chemicals (green *chemistry*) [11].

Purple spinach (*Alternanthera brasiliana)* is a wild plant that lives in empty gardens or roadside and it is not consumable like spinach. This research uses the *Alternanthera brasiliana* extract as a color indicator of urea biosensors. The leaf was extracted and tested as a urea biosensor indicator, some of the properties of the extract will be studied such as the effect of temperature, storage, pH & oxidizing agents.

MATERIALS AND METHODS

Materials

The materials used in this study including the purple spinach leaves obtained from Purwokerto, further identified their taxonomy in the Plant Taxonomy Laboratory, Faculty of Biology, Universitas Jenderal Soedirman. Urease from *Canavalia ensiformis* (Jack bean) Type III, powder, 15,000-50,000 units/g solid were purchased from Sigma (Germany). Urea, Ethanol, hydrochloric acid, distilled water, potassium chloride, citric acid, sodium citrate, disodium hydrogen phosphate, and sodium dihydrogen phosphate. All chemicals used were analytical grade purchased from Merck (Germany).

Apparatus and measurements

The instruments used in this research were UV- *Vis spectrophotometer* (Shimadzu Biospec 1601, Japan), magnetic *stirrer*, pH meter (Hanna instrument), and blender (Philips).

Leaf extraction

Purple Spinach leaves (*Alternanthera brasiliana*) have been separated from the stem and washed using tap water. Twenty grams of the wet clean stem was then crush using a blender. Purple spinach leaf slurry was then extracted using two different solvents distilled water and ethanol-HCl (ratio 85:15, ethanol 96% and HCl 1.5N). The ratio of sample and solvent was 1:5 (w/v). The mixture was stirred for 1 hour, precipitated for 30 minutes and filtered using filter paper. The extract was then measured using a spectrophotometer scanning from 400 to700 nm for maximum wavelength determination. The extract was diluted when the absorption was too high (A>1), to get the optimum absorbance of 0.2-1.0.

Effect of pH

The extract was tested for stability of color change at various pH (pH 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10). Two mL extract dissolved in 100 mL buffer according to the pH variation. The absorbance was measured at 400-700 nm using a spectrophotometer.

Effect of oxidizing agents

Ten mL of the extract was put into a test tube and 1 mL of 30% H_2O_2 as an oxidizing agent was added. In another tube, 10 ml of the solution was added, and 5 mg/mL of ascorbic acid was added as a reducing agent. Each tube was stored at room temperature for 12 hours. Every 3 hours, the absorbance of the extract was measured at 400-700 nm to study the effect of oxidizing and reducing agents. A blank sample as control was also prepared without the addition of reducing / oxidizing agents.

Heat effect on the extract color stability

Ten mL of the extract was prepared in a tightly closed bottle, then heated at 100°C for 1 and 2 hrs. The absorbance was measured every 1 h at a wavelength of 400-700 nm using a spectrophotometer.

Storage effect of the extract stability

The concentrated extract was stored at room temperature and a cold temperature (0-4℃) for 7 days. The color changes have been measured from day 0 to day 7. Before measurement, 2 ml concentrated extract was dissolved in 100 mL distilled water. The absorbance was measured every day at a wavelength of 400-700 nm.

Urea determination using leaf extract

Six tubes contain distilled water of 4.5 ml has been added 0.1M urea with various amount 0, 20, 40, 60, 80, 100 µl. Each tube was then added with the urease enzyme of 20 µl (1U/µl) and incubated for 30 minutes at room temperature. Purple spinach extract of 500 µl was then added to each sample tube. The color change was then measured at 400-700 nm using a spectrophotometer. The obtained data were then calculated to get the linear regression line of color change with the increase of urea concentration.

RESULTS AND DISCUSSION

Extraction of Purple Spinach (*Alternanthera Brasiliana*) Leaf

The purple spinach used has been identified in the Plant Taxonomy Laboratory and the results showed that the purple spinach used was a family of *Althernanthera*, genus species of *Alternanthera brasiliana*. The extraction of the purple spinach leaf using ethanol-HCl (85:15) had a deep purple while the extract with distilled water showed a dark red color (**Figure. 1**). Purple spinach leaf extract was measured using a spectrophotometer UV- *Vis* at the wavelength of 400-700nm, however, the extract obtained is too concentrated with high absorbance, thus the dilution required to obtain an absorbance between 0.2 - 0.8. The results showed that 10 times dilution of spinach leaf extract was required to obtain the desired absorbance. The extract of each solvent was measured using a UV- *Vis spectrophotometer* at 400-700 nm. The results showed the water extract had 2 peaks of 535 nm and 437, while the ethanol extract showed a *peak* at 526 (**Figure. 1C**)

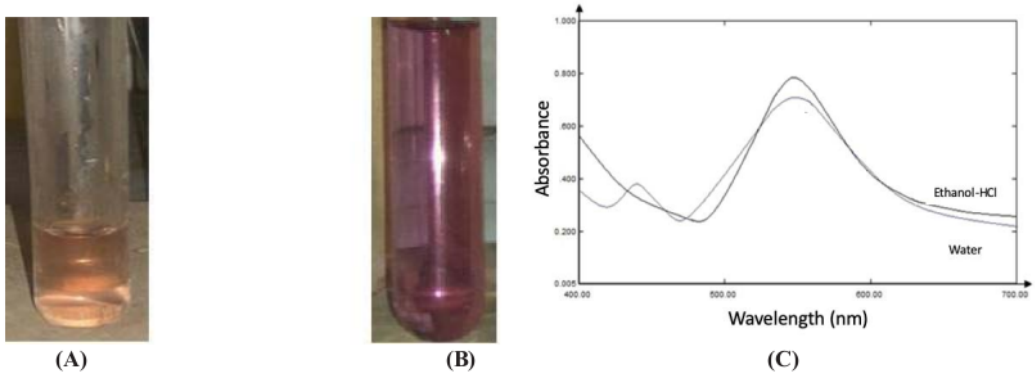

FIGURE 1. Purple spinach leaf extract in distilled water (A) and ethanol-HCl (B). The spectrum of water extract showed two peaks while ethanol-HCl extract only one peak (C).

The red and orange natural extracts were detected at 475-560 nm probably contains cyanidine 3-glycoside[12], similar to natural color extract from *Etlingera elatior* with the maximum absorbance at 523 nm [10]. The results obtained from this study showed the ethanol extracts were better than water extract since they had only one peak. However, it needs further characterization to justify the best solvent for purple spinach leaf extraction.

Extract stability study

The purple spinach leaf extract which was obtained using distilled water and ethanol-HCl were then studied for the stability of the extracted color with various environmental effects such as the effect of storage time, the effect of heating, the effect of pH, and the effect of oxidizing-reducing agents.

Effect of storage time

Water extract of purple spinach color was changed from red color brown to greenish-brown while the ethanol-HCl extract color was also changed from dark purple to colorless for less than 7 days of storage at room temperature. Storage spinach extract in cold temperatures showed better color stability than the room temperature (**Figure. 2**). Color degradation could be caused by the decomposition of anthocyanin structure of the cation flavylium red, become hemiacetal or colorless carbinol base, and eventually became colorless chalcone [13]. Elevated temperatures cause the decomposition of anthocyanin structure was faster than the cooler temperature. The decrease in absorbance at room temperature was faster than at cool temperatures, because at high temperatures it can cause faster color degradation which also reduces absorbance.

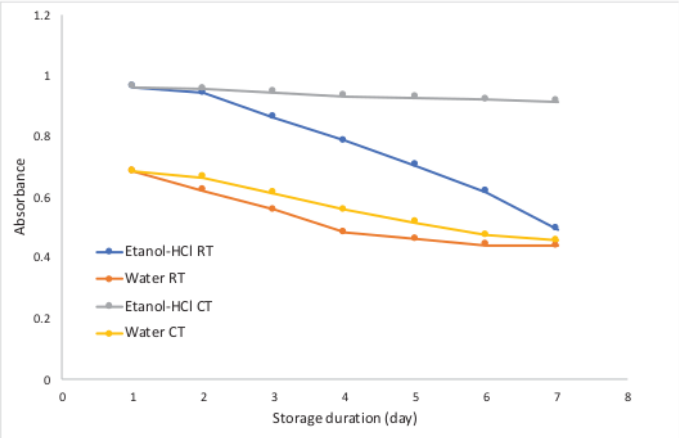

FIGURE 2. Effect of storage duration of the purple spinach leaf extract at room temperature (RT) and cool temperature (CT)

Effect of pH

The extract needs certain conditions to maintain its stability, for example, was pH of the solution. Purple spinach leaf extract in buffer solution pH of 3, 4, and 5 had dark purple (ethanol-HCl) and dark red (water) color. At pH 6 and 7, it has a less intense purple color (ethanol-HCl) and brownish red (water). At pH 8, and 9 have a pale purple color to colorless (ethanol-HCl) and greenish-brown (water) (**Figure. 3**). Anthocyanins at pH more than 2 there tend to rapid reduction of protons to red or quinoidal blue which then flavylium cations become hydrated to produce colorless carbinol or become colorless chalcone forms [14].

Effect of oxidizing agents

Studying the effect of oxidizing agents on the absorbance of spinach extract was performed to determine other conditions generally found in the application of natural extract. The oxidizing agent selected in this treatment was hydrogen peroxide, which was a weak acid and highly reactive, while the reducing agent used was ascorbic acid (vitamin C). The effect of the oxidizing agent had reduced the color intensity for both ethanol and water extract. On another side, the reducing agent was only slightly reducing the color intensity of both ethanol-HCl extract and water extract (**Figure. 4**). The behavior of the ethanol-HCl extract for stable in in the present of reducing agent and it was not stable in the present of oxidizing agent similar to previously report of *Etlingera elatior* extract [10]. Oxidizing

agents reduce the color of the extract since the red flavylium loses their protons and turn into carbonyl which was colorless.

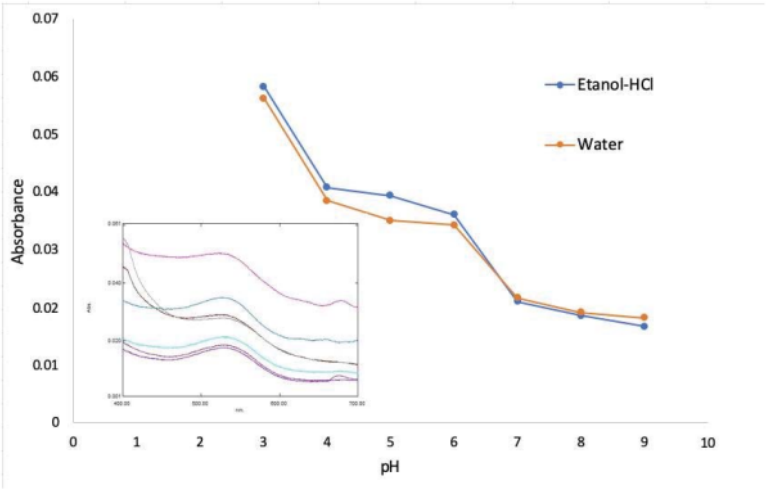

FIGURE 3. Effect of pH on the absorbance of the purple spinach leaf extract with ethanol-HCl and distilled water. Inset the spectrum change with the increase of the pH.

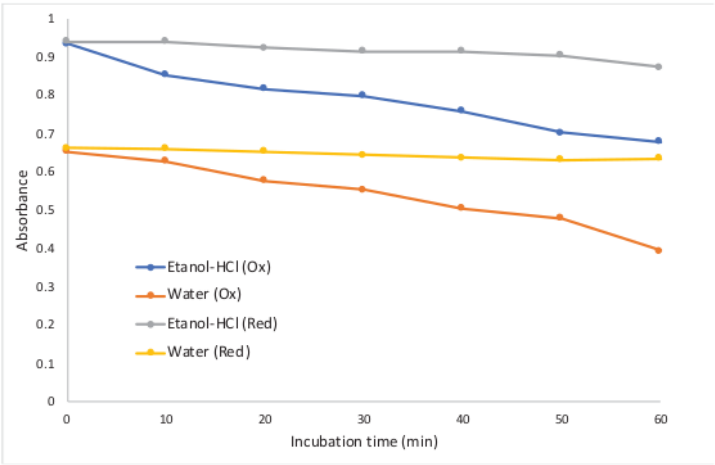

FIGURE 4. Effect of oxidizing agent (Ox) and reducing agent (Red) on the ethanol-HCl and water extract with the incubation time up to 60 minutes.

Effect of heating

This study was carried out by varying the heating time of the extract at 100 ^{0}C for 15 minutes. The absorbances of the extracts were recorded at 0, 3, 6, 9, 12, and 15 minutes of heating. The heat treatment significantly reduces the absorbance of both ethanol-HCl extract and water extract (**Figure. 5**). The heat treatment may damage the structure of anthocyanin, by hydrolysis of the three glycosidic bonds of anthocyanins to produces labile aglycones or ring opening to form a colorless carbonyl group [15].

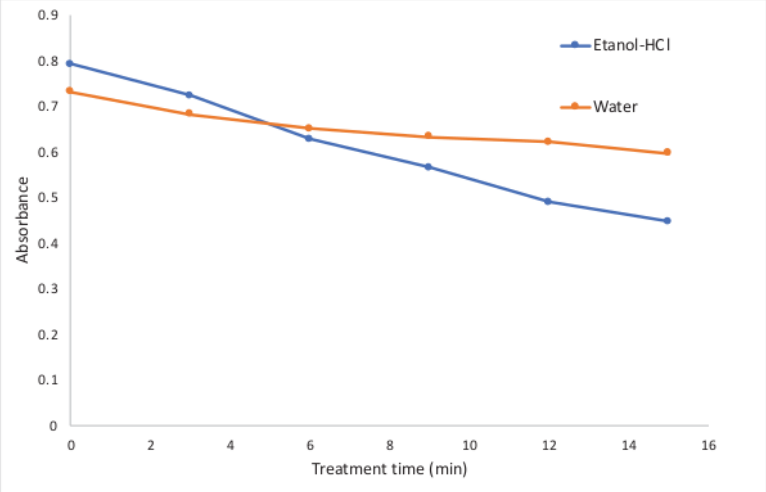

FIGURE 5. Effect of heat treatment on the stability of ethanol-HCl and water extract of purple spinach leaf

Purple spinach extract study as a urea biosensor indicator

The extract was tested as a urea biosensor indicator using the enzymatic reaction of urea and urease enzyme. This biosensor indicator was used to determine color changes with the increase of urea concentration. The result showed a linear response for both ethanol-HCl and water extract with various urea concentrations 0, 10, 20, 30, 40, and 50 mM (**Figure. 6**). The urea concentration showed a negative correlation with the absorbance, since the higher concentration of urea would reduce the red color of anthocyanins, which was stable acidic conditions, into colorless alkoxy in alkaline conditions and affect the decrease in absorbance [13]. The urea standard curve showed a linear response of 0-50µM. The absorbance of the standard curve was then calculated to get the regression equation of $y = -0.0011x + 0.824$ ($R^2 = 0.965$) for ethanol-HCl extract and $y = -0.001x + 0.640$ ($R^2 = 0.923$) for water extract. These results showed that the purple spinach extract could be used as an indicator of the urea biosensor.

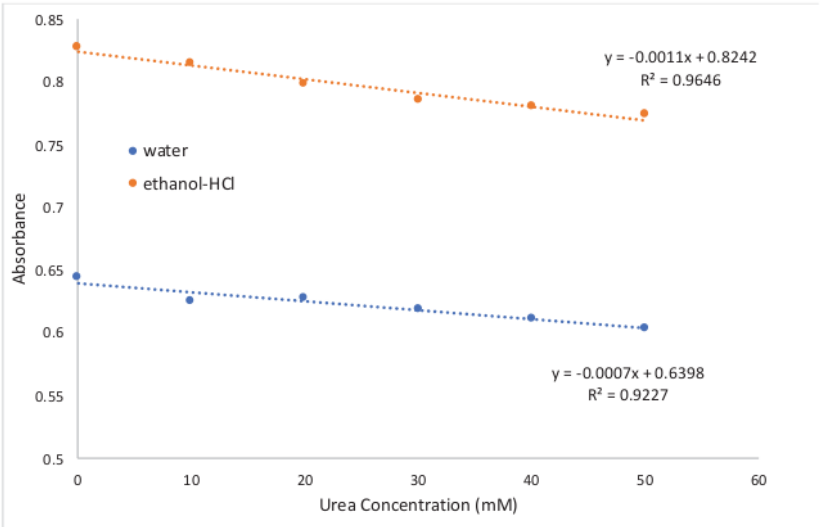

FIGURE 6. Standard calibration curve of urea using purple spinach leaf water extract and ethanol-HCl extract.

CONCLUSION

The solvent for the purple spinach (*Alternanthera brasiliana*) leaves extraction using ethanol-HCl showed a better result than that of distilled water. The characterization results showed that purple spinach leaves extract color intensity was decreased with the heating, oxidizing agents, storage at room temperature and alkaline condition. Both ethanol-HCl and water extract of purple spinach leaf showed linear responses for urea determination with the ethanol-HCl extract showed better sensitivity than the water extract for urea determination in the range of 10 to 50 mM.

ACKNOWLEDGEMENT

We would like to thank the Directorate General of Higher Education (DGHE, DIKTI) and the Jenderal Soedirman University for supporting this research through "Riset Unggulan Perguruan Tinggi" grant No 068/SP2H/LT/DRPM/IV/2017

REFERENCES

1. D.L. Nelson, A.L. Lehninger, and M.M. Cox, *Lehninger Principles of Biochemistry* (Macmillan, 2008).
2. M. Rahmatullah and T.R.C. Boyde, *Clin. Chim. Acta* **107**, 3 (1980).
3. Z. Zusfahair, D.R. Ningsih, E.D.P. Lestari, and A. Fatoni, *Molekul* **14**, 64 (2019).
4. U. Thanei-Wyss, W.E. Morf, P. Lienemann, Z. Stefanac, I. Mostert, R. Dörig, R.E. Dohner, and W. Simon, *Microchim. Acta* **81**, 135 (1983).
5. A. Fatoni, A.N. Aziz, and M.D. Anggraeni, *Sens. Bio-Sensing Res.* **28**, (2020).
6. Amin Fatoni, M.D. Anggraeni, and D.W. Dwiasi, *J. Anal. Chem.* **74**, (2019).
7. C. Griesche and A.J. Baeumner, *TrAC Trends Anal. Chem.* **128**, 115906 (2020).
8. A. Fatoni, Zusfahair, S. Nurfiah, M.D. Anggraeni, and A.N. Aziz, in *AIP Conf. Proc.* (AIP Publishing, 2019), p. 20021.
9. A. Fatoni, M.D. Anggraeni, and L.Z. Zulhidayah, in *IOP Conf. Ser. Mater. Sci. Eng.* (IOP Publishing, 2019), p. 12010.
10. A. Fatoni, M.D. Anggraeni, Z. Zusfahair, and H. Iqlima, in *IOP Conf. Ser. Mater. Sci. Eng.* (IOP Publishing, 2018), p. 12015.
11. P.T. Anastas, *Crit. Rev. Anal. Chem.* **29**, 167 (1999).
12. L. Simanjuntak and F. Chairina Sinaga, *J. Tek. Kim. USU* **3**, (2014).
13. D.R. Adawiyah, (n.d.).
14. G. Mazza and E. Miniati, *Anthocyanins in Fruits, Vegetables, and Grains* (CRC press, 2018).
15. J. He, Isolation of Anthocyanin Mixtures from Fruits and Vegetables and Evaluation of Their Stability, Availability and Biotransformation in the Gastrointestinal Tract, The Ohio State University, 2008.

8. A green chemistry approach

ORIGINALITY REPORT

10%	%	%	10%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

10%

★ Submitted to Universitas Mataram

Student Paper

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		