IOP Conference Series: Earth and Environmental Science

Selection of potential Indonesian plant species for antioxidant

Selection of potential Indonesian plant species for antioxidant

K Kartini[1]*, F Setiawan[1], J Sukweenadhi[2], O Yunita[1] and C Avanti[3]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia
[2]Faculty of Technobiology, University of Surabaya, Surabaya, Indonesia
[3]Department of Pharmaceutic, Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia

*Corresponding author: kartini@staff.ubaya.ac.id

Abstract. Diabetic foot ulcer (DFU) is commonly complication of diabetes mellitus. Antimicrobial and antioxidant herbs can be used as complementary therapy in DFU and these properties will be strengthened by silver nanoparticles (AgNPs). This study was performed for screening of Indonesian herbs promising as antioxidant which can be subsequently involved in the green synthesis of AgNPs. Antioxidant of eight herbs extracts were evaluated using 1,1-diphenyl-2-picrylhydrazyl method. Their total group of compounds were quantified as well. *Phyllanthus niruri* and *Orthosiphon stamineus* exhibited the strongest antioxidant power with IC_{50} of 102 and 133 µg/ml, followed by *Curcuma domestica, Stelechocarpus burahol,* and *Curcuma xanthorriza* with the IC_{50} of 363, 481, and 540 µg/ml, respectively. *Sonchus arvensis, Apium graveolens, Centella asiatica* did not have antioxidants activity. Total phenolics from the highest is: *O. stamineus* > *C. domestica* > *C. xanthorrizha* > *P. niruri* > *S. burahol* > *S. arvensis* > *A. graveolens* > *C. asiatica,* whereas total flavonoids order is *O. stamineus* > *S. burahol* > *P. niruri* > *A. graveolens* > *C. asiatica* > *S. arvensis* > *C. xanthorrizha* > *C. domestica*. We concluded that *O. stamineus* was the most suitable herb to be used as a bioreductor in the green synthesis of AgNPs.
Keywords: Antioxidant, silver nanoparticles, diabetic wound healing, medicinal plants

1. Introduction

A high prevalence of diabetes causes the emergence of comorbidities. The critical effects of hyperglycemia include micro-vascular and macro-vascular complications [1]. Diabetes is the cause of non-traumatic lower limb amputation, which is preceded by wounds that do not heal [2]. The risk of wound in diabetics is 15-20% [3]. About 15-27% of diabetic patients experience amputation due to infection [4]. 45-60% of diabetic foot ulcers (DFU) are estimated due to neuropathy [5]. Emerging studies show high infections of *Pseudomonas* [6], *E. coli* [7], and *S. aureus* [8] in DFU. Diabetic foot ulcers treatment requires handling of wounds as well as giving appropriate antibiotics [1]. Silver/sulphadiazine dressing can be considered in DFU therapy. Silver and polyherbal dressing shows promising results in wound healing [9].

Various plant species have been used empirically in Indonesia for wound healing [10]. Some potential species for DFU therapy are *Curcuma xanthorrhiza* (*temulawak*), *Curcuma domestica* (*kunyit*), *Centella asiatica* (*pegagan*), *Stelechocarpus burahol* (*kepel*), *Orthosiphon stamineus* (*kumis kucing*), *Phyllanthus niruri* (*meniran*), *Sonchus arvensis* (*tempuyung*), and *Apium graveolens* (*seledri*)

[11]. These plants contain antioxidant and antimicrobial compounds, as well as being able to reduce metals [12-14].

Nanoparticles are the current technology and have been used in many fields, one of which is metal nanoparticles [2]. It has been widely used in biomedical and physicochemical fields [2]. Silver nanoparticles are also widely used for antimicrobial, anticancer, anti-inflammatory, and wound treatment [15, 16]. Green synthesis of nanoparticles is carried out using microorganisms, plants, yeast, or viruses. Nanoparticle synthesis using plants, phytonanotechnology, has a lot of advantages such as biocompatibility, fast, stable, environmentally friendly, affordable, and easy to scale-up [2]. Various chemical compounds including primary metabolites such as protein and polysaccharides as well as secondary metabolites i.e. terpenoids, phenolics, flavonoids, and alkaloids can be applied in the green synthesis of metal nanoparticles including silver nanoparticles (AgNPs) [17]. They work as reducing and capping agents [18]. The green synthesis of AgNPs involving plant extracts can be an option to increase the effectiveness of topical preparations for DFU treatment [19]. As an initial step, this research was conducted to select native Indonesian plant species having potential as antioxidants. Selected plants will be further developed for the green synthesis of silver nanoparticles as DFU treatment.

2. Material and methods

2.1. Plant species

This research used eight plant species as described at table 1. All crude drugs were obtained from and authenticated by Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) located at Tawangmangu, Indonesia.

Table 1. Selected Indonesian plant species for antioxidant.

Plant species	Part used
Orthosiphon stamineus	Leaves
Curcuma domestica	Rhizome
Curcuma xanthorriza	Rhizome
Phyllanthus niruri	Herbs
Sonchus arvensis	Leaves
Apium graveolens	Herbs
Centella asiatica	Herbs
Stelechocarpus burahol	Leaves

2.2. Extraction

Dried plants were milled into 2 mm before used. Each powdered plant was then refluxed with aqueous ethanol for 3 x 2.5 h. The ethanol were then removed from the extracts by using rotary evaporator at the temperature of 40°C, continued by evaporation on waterbath to yield a thick extract. The thick extracts were subsequently stored in a desiccator.

2.3. Quantification of total phenolics

Concentration of the total phenolics in the crude extracts was determined by using Folin-Ciocalteu (FC) reagent according to the previous studies [20, 21]. Analysis was carried out on 96-well microplates by using gallic acid as a standard. It was prepared in various concentrations started from 2 to 10 µg/ml. Reaction was initiated by adding FC reagent (0.1 mol/l, 150 µl), followed by 30 µl of each ethanol extract or standard solution. After 10 min, solution of Na_2CO_3 (7.5%, 120 µl) was added and incubated for two hours. The absorbance was subsequently read at the wavelength of 760 nm. Total phenolics concentration of the extracts was calculated by interpolation of the absorbance to the calibration curve of standard. The total phenolics concentration was presented as gram of gallic acid equivalents (GAE) per 100 g of thick extract.

2.4. Quantification of total flavonoids

Evaluation of total flavonoids concentration was conducted by established colorimetric methods as detailed in previous study [22]. Thick extract was dissolved and diluted in 96% ethanol. One ml of extract was transferred into a volumetric flask, supplemented with 4 ml of demineralized water and solution of $NaNO_2$ (5%, 0.3 ml), followed by incubation at room temperature for 5 minutes. Solution of $AlCl_3$ (10%, 0.3 ml) was then added at the 6th minute. Sodium hydroxide (1 M, 2 ml) and demineralized water were finally added up to 10.0 ml. The absorbance was subsequently read at the wavelength of 400.2 nm. Total flavonoids concentration of the extracts was calculated by interpolation of the absorbance to the calibration curve of standard (quercetin, 5 to 25 µg/ml). The total flavonoids concentration was expressed as gram of quercetin equivalents (QE) per 100 g of thick extract.

2.5. Antioxidant activity

Antioxidant activity test referred to the previous work with slight modification [23]. It was based on the capability of the extract to scavenge free radical of DPPH. In summary, the method is as follows: solution of DPPH in methanol (0.026%, 50 µl) was pipetted to 100 µl of serial dilution of extracts in methanol (total volume 150 µl). It was left in darkness for 15 min and the absorbance was then measured at the wavelength of 517 nm using a microplate reader. Free radical scavenging activity was calculated using the following formula:

where, A_B = absorption of DPPH solution (t = 0 min); A_A = absorption of tested samples (t = 15 min). IC_{50} value, the concentration of extract, which exhibited 50% radical scavenging activity, was deduced from the linear regression of concentration versus percentage of inhibition [24].

2.6. Statistical analysis

Both total phenolics/flavonoids and IC_{50} values were presented as average ± SD, n = 3 or 4. Data were statistically analysed using ANOVA followed with post hoc Tukey test. All analysis was conducted with the statistical software, GraphPad Prism (GraphPad Software Inc. Windows Version 5.01). Differences were concluded to be significant at P value of less than 0.05.

3. Results and discussion

3.1. Total phenolics and flavonoid concentration

Group of compounds, phenolics and flavonoids, have been determined in this research to evaluate the quality of each herb material. Total phenolics was analysed with the Folin-Ciocalteu method, whereas total flavonoids was evaluated with the aluminum chloride ($AlCl_3$) colorimetric method using spectrophotometer. A series concentration of gallic acid (figure 1) as well as quercetin (figure 2) were prepared to set up the standard calibration curves, which were then applied to quantify the total phenolics and flavonoid contents in each crude extract. Table 2 presents total phenolics content as well as total flavonoid compounds calculated for each plant extract. It could be observed that phenolics and flavonoid contents of *Orthosiphon stamineus* extract were the highest.

Table 2 demonstrate that total phenolics content from the highest is as follow: *O. stamineus* > *C. domestica* > *C. xanthorrizha* > *P. niruri* > *S. burahol* > *S. arvensis* > *A. graveolens* > *C. asiatica,* whereas the total flavonoid content order is *O. stamineus* > *S. burahol* > *P. niruri* > *A. graveolens* > *C. asiatica* > *S. arvensis* > *C. xanthorrizha* > *C. domestica*. *Orthosiphon stamineus* contains the highest phenolics and flavonoids.

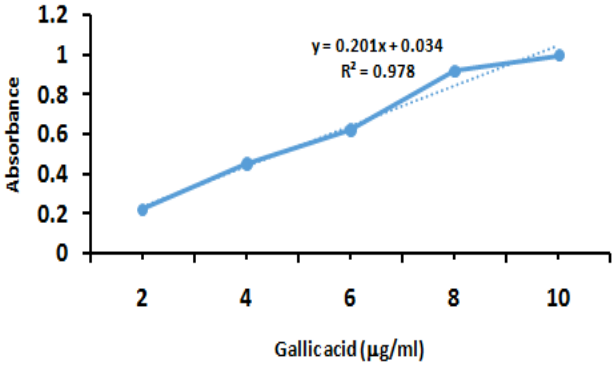

Figure 1. Calibration curve of gallic acid

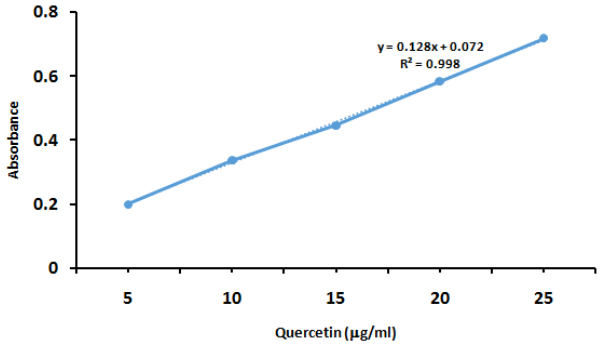

Figure 2. Calibration curve of quercetin

Table 2. Total phenolics and flavonoids concentration of selected Indonesian plant species extracts.

Plant species	Total phenolics concentration (g of GAE/100 g of extract)*	Total flavonoids concentration (g of QE/100 g of extract)**
Orthosiphon stamineus	44.91 ± 0.24^{a}	20.03 ± 0.68^{a}
Curcuma domestica	13.10 ± 0.67^{b}	1.24 ± 0.48^{b}
Curcuma xanthorriza	7.38 ± 0.40^{c}	2.77 ± 1.58^{b,c}
Phyllanthus niruri	7.22 ± 0.06^{c}	8.34 ± 0.74^{d}
Sonchus arvensis	2.62 ± 0.15^{d}	3.78 ± 0.09^{c}
Apium graveolens	2.16 ± 0.05^{d}	6.67 ± 0.31^{d}
Centella asiatica	0.67 ± 0.03^{e}	4.06 ± 0.27^{c}
Stelechocarpus burahol	5.85 ± 0.18^{f}	9.74 ± 0.27^{e}

*,**Values in the column represent average ± SD of 3 determinations. Averages followed by different notation within same column show significant differences ($P < 0.05$).

Flavonoids such as 3'-hydroxy-5,6,7,4'-tetramethoxyflavone, sinensetin, as well as eupatorin have been identified from *O. stamineus,* in addition to rosmarinic acid, a caffeic acid derivative. Moreover, total phenolics and flavonoids of this crude drug varies according to the geographical origin [25]. Phenolics content of *O. stamineus* correlates with the solvent, duration, and temperature of extraction. The optimum conditions for phenolics extraction from *O. stamineus* were 40% alcohol for 120 min at 65°C [26].

3.2. Antioxidant activity

Antioxidant activity of eight Indonesian plant species has been determined with free radical scavenging method against stable DPPH (1,1-diphenyl-2-picrylhydrazyl). DPPH is a commonly used free radical in the antioxidant screening assay. It has an unpaired electron from nitrogen atom. When a

compound or material with capability to donate hydrogen is reacted with the DPPH, it will reduce DPPH become DPPH-H (1,1-diphenyl-2-picrylhydrazyn) (figure 3) [27].

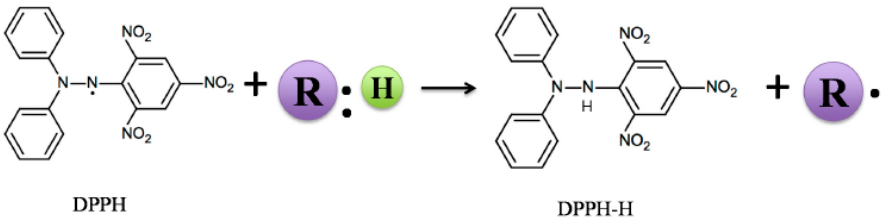

Figure 3. Reduction mechanism of 1,1-diphenyl-2-picrylhydrazyl (DPPH), R:H = antioxidant component; R• = antioxidant radical [27].

DPPH is a crystal with violet color. Its solution in ethanol absorbs UV light at the wavelength of 519 nm. If a phytochemical is able to donate hydrogen atom, it will scavenge the DPPH and this is indicated by the changing of the solution color, from violet to yellow, and the absorption will be lower as well. The extent of decrease in DPPH absorption is directly proportional to the concentration of radicals that are being inhibited. Assay was simply performed using a UV-visible spectrophotometer at ambient temperature, and the antioxidant capacity is represented as the percentage of DPPH radical inhibition. Figure 4 shows the correlation between the concentrations of *O. stamineus* and *P. niruri* extracts with the DPPH radical inhibition. This graph reveals that the higher the concentration of the plant extracts, the higher the inhibition of DPPH.

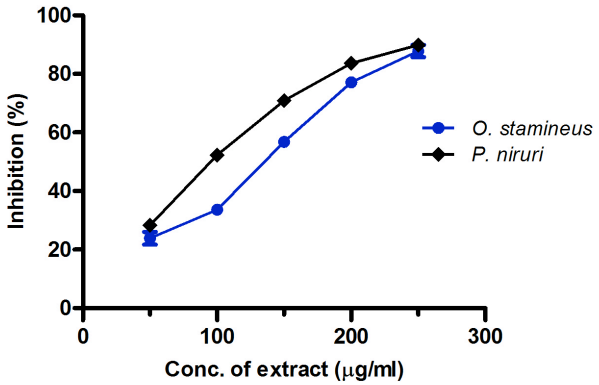

Figure 4. DPPH radical inhibition of various concentration of *O. stamineus* and *P. niruri* extracts.

The DPPH radical scavenging method is based on two reactions i.e. electron transfer as well as hydrogen atom transfer. This assay is widely used than other non-enzymatic antioxidant assay since its simplicity, rapidity, and low cost. To compare the antioxidant activity among the extracts, IC_{50} values (Table 3) were calculated based on the linear regression of concentration (µg/ml) versus inhibition (%).

Table 3 reveals that *O. stamineus* and *P. niruri* are the most active extracts as a radical scavenger. The IC_{50} value is one parameter to classify the potency of an antioxidant. According to Jun et al. [28], antioxidant activity is classified into 5 groups: highly active (IC_{50} < 50 µg/ml), active (IC_{50} = 50-100 µg/ml), moderate (IC_{50} = 101-250 µg/ml), weak (IC_{50} = 250-500 µg/ml), and inactive (IC_{50} > 500 µg/ml). Table 2 indicates that *O. stamineus* and *P. niruri* have moderate antioxidant activity, whereas the others are weak and inactive antioxidants.

Table 3. IC_{50} value of DPPH scavenging activity by selected extracts of Indonesian plant species.

Extract of plant species	IC_{50} (µg/ml)
Orthosiphon stamineus	133 ± 6.6[a]
Curcuma domestica	363 ± 9.7[b]
Curcuma xanthorriza	540 ± 12.8[c]
Phyllanthus niruri	102 ± 1.7[a]
Sonchus arvensis	1135 ± 7.1[d]
Apium graveolens	2263 ± 61.8[e]
Centella asiatica	ND
Stelechocarpus burahol	481 ± 2.7[f]
Ascorbic acid*	31 ± 1.6
Trolox*	76 ± 1.1

*standard compound; ND: not detected; Values in the column represent average ± SD of IC_{50} (inhibitory concentration 50, n = 4). Averages followed by different notation within same column show significant differences ($P < 0.05$).

Flavonoids and phenolics are ubiquitous compounds which can be found in many species of plants. They are main source of natural antioxidants in plants, fruits, and vegetables. In addition to flavonoids especially lipophilic flavones as well as caffeic acid derivatives, *O. stamineus* contains terpenoids and essential oil. Lipophilic flavones and caffeic acid ester of 3-(3,4-dihydroxyphenyl)lactic acid were main phenolics compounds found in the crude extract of *O. stamineus* and their antioxidant activities have been well determined [29, 30]. However, in our research, there was no linear correlation between the antioxidant capacity and the total phenolics concentration ($R^2 = 0.238$) as well as between antioxidant power and the total flavonoids concentration ($R^2 = 0.079$). The same pattern on the correlation between antioxidant capacity of plant extracts and the phenolics concentration have been previously investigated ($R_{xy} = 0.048$) [25, 31]. Phenolics is not the only phytochemical with antioxidant properties. The existence of various other anti-oxidant compounds in the plant extracts, which are able to donate hydrogen may erroneously contribute to the content of the total phenols determined with Folin–Ciocalteau reagent. Therefore, it is no simple correlation between the level of total phenolics and the antioxidant activity when compare among herb materials [25].

4. Conclusion

Phyllanthus niruri and *Orthosiphon stamineus* showed the most potent antioxidant with an IC_{50} value of 102 and 133 µg/ml, followed by *C. domestica, S. burahol,* and *C. xanthorriza* with the IC_{50} value of 363, 481, and µg/ml ppm, respectively. *Sonchus arvensis, A. graveolens,* and *C. asiatica* exhibited poor antioxidant capacity with the IC_{50} value > 1000 ppm. Total phenolics content from the highest is as follow: *O. stamineus* > *C. domestica* > *C. xanthorrizha* > *P. niruri* > *S. burahol* > *S. arvensis* > *A. graveolens* > *C. asiatica,* whereas the total flavonoids content order is *O. stamineus* > *S. burahol* > *P. niruri* > *A. graveolens* > *C. asiatica* > *S. arvensis* > *C. xanthorrizha* > *C. domestica*. *Orthosiphon stamineus* contains the highest concentration phenolics and flavonoids. *Orthosiphon stamineus* was the most suitable herbs to be used as a source of bioreductors in the green synthesis of silver nanoparticles for foot ulcer.

Acknowledgements

We thank to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia who has supported this work under Fundamental Research Scheme with the contract number: 004/SP2H/LT/MULTI/L7/2019.

References

[1] Clayton Jr W and Elasy T A 2009 *Clin. Diabetes* **27** 52
[2] Kavitha K V, Tiwari S, Purandare V B, Khedkar S, Bhosale S S and Unnikrishnan A G 2014 *World J. Diabetes* **5** 546
[3] Singh P, Kim Y-J, Zhang D and Yang D-C 2016 *Trends Biotechnol.* **34** 588
[4] Reiber G E 2002 *The Diabetic Foot* (Netherland: Springer Netherlands)
[5] Richard J-L, Sotto A and Lavigne J-P 2011 *World J. Diabetes* **2** 24
[6] Shanmugam P and Jeya M 2013 *J. Clin. Diagn. Res.* **7** 441
[7] Tiwari S, Pratyush D D, Dwivedi A, Gupta S K, Rai M and Singh S K 2012 *J. Infect. Dev. Ctries.* **6** 329
[8] Abdulrazak A, Bitar Z I, Al-Shamali A A and Mobasher L A 2005 *J. Diabetes Complications* **19** 138
[9] Viswanathan V, Kesavan R, Kavitha K and Kumpatla S 2011 *Indian J. Med. Res.* **134** 168
[10] Elfahmi, Woerdenbag H J and Kayser O 2014 *J. Herb. Med.* **4** 51
[11] Wientarsih I, Winarsih W and Sutardi L N 2013 *Jurnal Veteriner* **13** 251 (*In Indonesian Language*)
[12] Shameli K, Ahmad M B, Zamanian A, Sangpour P, Shabanzadeh P, Abdollahi Y, et al 2012 *Int. J. Nanomedicine* **7** 5603
[13] Logeswari P, Silambarasan S and Abraham J 2015 *J. Saudi Chem. Soc.* **19** 311
[14] Singh K, Panghal M, Kadyan S and Yadav J P 2014 *JNMNT* **5** 1
[15] Singh N, Armstrong D G and Lipsky B A 2005 *Jama* **293** 217
[16] Keat C L, Aziz A, Eid A M and Elmarzugi N A 2015 *Bioresour. Bioprocess.* **2** 47
[17] Ahmed S, Ahmad M, Swami B L and Ikram S 2016 *J. Adv. Res.* **7** 17
[18] Duan H, Wang D and Li Y 2015 *Chem. Soc. Rev.* **44** 5778
[19] Mishra M, Kumar H and Tripathi K 2008 *Dig. J. Nanomater. Bios.* **3** 49
[20] Beara I N, Lesjak M M, Jovin E Đ, Balog K J, Anačkov G T, Orčić D Z et al 2009 *J. Agric. Food Chem.* **57** 9268
[21] Singleton V L, Orthofer R and Lamuela-Raventos R M 1999 *Methods Enzymol.* **299C** 152
[22] Chang C-C, Yang M H, Wen H M and Chern J C 2002 *J. Food Drug Anal.* **10** 178
[23] Galvez M, Martin-Cordero C, Houghton P J and Ayuso M J 2005 *J. Agric. Food Chem*. **53** 1927
[24] Chen Z, Bertin R and Froldi G 2013 *Food Chem.* **138** 414
[25] Akowuah G, Zhari I, Norhayati I, Sadikun A and Khamsah S 2004 *Food Chem.* **87** 559
[26] Chew K, Khoo M, Ng S, Thoo Y, Aida WW and Ho C 2011 *Int. Food Res. J.* **18** 1427
[27] Liang N and Kitts D 2014 *Molecules* **19** 19180
[28] Jun M, Fu H Y, Hong J, Wan X, Yang C and Ho CT 2003 *J. Food Sci.* **68** 2117
[29] Brand-Williams W, Cuvelier M E and Berset C 1995 *LWT - Food Sci. Technol.* **28** 25
[30] Cuvelier M-E, Richard H and Berset C 1992 *Biosci. Biotechnol. Biochem.* **56** 324
[31] Maillard M-N and Berset C 1995 *J. Agric. Food Chem*. **43** 1789