JOURNAL OF SUSTAINABLE NATURAL RESOURCES VOL. 1 NO. 2 (2020) 32-36

J-SuNR

Journal homepage: http://publisher.uthm.edu.my/jsunr

e-ISSN : 2716-7143

Journal of Sustainable Natural Resources

Characterisation of *Asystasia Gangetica* and *Phyllanthus Niruri* Extracts: Total Phenolic Content, Antioxidant and Antibacterial Activities

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DOI: https://doi.org/10.30880/jsunr.2020.01.02.006
Received 25 November 2019; Accepted 22 December 2020; Available online 24 December 2020

Abstract: Antioxidant plays a significant role in inhibiting and scavenging free radicals, which protects human against infection and degenerative diseases. Nowadays, most studies focus on "natural antioxidants" from herbs due to safe therapeutic. In this study, performance on different extraction methods, i.e. Soxhlet, cold maceration and traditional extraction method assisted with ultrasonic-assisted extraction (UAE) for *Asystasia gangetica* leaves and *Phyllanthus niruri* plant are investigated by means of antioxidant, total phenolic content and antibacterial activity. The extracts were characterized for its antioxidant activity and total phenolic content by means of DPPH radical scavenging method and Folin-Ciocalteau reagent respectively. Optimization studies were carried out on combined cold-maceration and UAE extraction method by using Response Surface Method. Then, the optimized extracts were further characterized for antibacterial activity using *Escherichia coli* and *Staphylococcus epidermidis* by the agar disc diffusion method. Sample containing cold macerated *A. gangetica* and *P. niruri* indicates the highest antioxidant property as it contains the highest percentage of DPPH scavenging activity (59.57% and 51.14% respectively). While, the total phenolic content of macerated *A. gangetica* and *P. niruri* are 2.73 mg GAE/g and 2.68 mg GAE/g respectively. Consequently, after the determination of highest percentage of DPPH scavenging activity, the extracts were further optimized using UAE. From the optimization, the optimum condition was determined to be power of 17.57±0.01W and time of 5.83±0.01minutes for *A. gangetica* while, the power of 98.14±0.01W and 0.17±0.01minutes for *P. niruri*. Both optimized extracts showed potent antibacterial activity with high degree of antibacterial against *E. coli*. In conclusion, combination of cold maceration and UAE method increase the amount of antioxidant from extracts of *A. gangetica* and *P. niruri*. The finding from this study can be useful in pharmaceutical and cosmeceutical industries.

Keywords: *Asystasia gangetica, Phyllanthus Niruri*, Antioxidant Activity, Total Phenolic Content, Antibacterial Activity.

1. Introduction

Malaysia is well known for its diversity in herbs [1]. Herbs is identified as a source of various phytochemicals that have a part in antioxidant defense. Study of herbs is important for making next effort towards standardization of plant checking as medicine. There are numbers of herbs in Malaysia used for medicinal purpose. For instance, *Tongkat ali* is used to treat erectile dysfunction.

Another medicinal herbs are Chinese Violet (*Asystasia gangetica*) is a species of Acanthaceae family used traditionally to treat disease such as diabetes. *Phyllanthus niruri* is a tropical shrub belonging to Phyllanthaceae family,

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commonly used traditionally, such as diarrhea and cough [2]. *P. niruri* and *A. gangetica* plant is easily grow, which may be found along the roadsides, in street corners and dumps of building materials (Paithankar [3]. *P. niruri* and *A. gangetica* contains phytochemical and pharmacological properties [4].

Herein, this study focuses on effect of different extraction methods i.e. Soxhlet extraction, cold maceration and traditional extraction assisted by ultrasonic extraction for extracts of *A. gangetica* and *P. niruri* plants. The extracts were characterized for antioxidant activity and the total phenolic content. The optimum condition for the ultrasonic-assisted extraction method were determined by using 'Design of Experiment' software. The optimized extracts were further characterized for antibacterial activity. The relationship between the antioxidant activity, total phenolic content and antibacterial activity of *A. gangetica* and *P. niruri* extracts were determined and analyzed for authors.

2. Materials and Methods

2.1 Plant Material

A. gangetica and *P. niruri* plants were collected directly from Nasuha Herbs & Spice Farm in Muar, Johor. The collected plant was first cleaned to remove any undesirable materials and contaminants under running water. Then, the leaves were dried in the oven at 44.5 ºC for 4 hours under forced-air ventilation [5]. The dried leaves were pulverized using blender to form coarse particles. These particles were stored in airtight containers.

2.2 Preparation of Crude Extracts

The coarse particles (10g) of both plants were extracted using three different methods i.e. Soxhlet, cold maceration and traditional methods. Ethanol was used as solvent for Soxhlet and maceration, while distilled water was used as solvent for traditional method with a constant volume of 150ml. Aqueous extracts were obtained by centrifugation at 10,000 rpm for 10 minutes. The extracts containing ethanol were concentrated on a rotary vacuum evaporator, [6] at 34ºC and 58 mbar.

2.3 Optimization using Response Surface Methodology (RSM)

Extraction method in which possess high percentage of DPPH radical scavenging was selected before further extracted by using ultra-sonication. In this study Central Composite design (CCD) of Response Surface Methodology (RSM) was performed using Design Expert Software to monitor effects of different modes of ultra-sonication operation in respect to power and extraction time. The responses of optimization are antioxidant activity and total phenolic content. The optimum condition was compared with combination of selected extraction (i.e. Soxhlet, cold maceration and traditional methods) and UAE method, and non-combined extraction method.

2.4 Determination of Antioxidant Activity

DPPH radical scavenging method was used to determine the antioxidant activity of the extracts from different extraction methods. 1 mL extract solution was transferred into different test tubes. Then, 5 mL of ethanolic solution of DPPH was added into the test tube [7]. The mixtures were shaken and incubated at room temperature for 30 minutes. The absorbance value of the prepared solution was measured using UV-VIS Spectrophotometer at 517 nm. Ethanol solution act as a blank and the absorbance of DPPH act as a control. Where, the percentage of antiradical activity was calculated by using following formula:

$$\% \text{ DPPH Radical scavenging} = \frac{\text{Control Abs-Abs Sample A}}{\text{Control Abs}} \times 100 \quad (1)$$

2.5 Determination of Total Phenolic Content (TPC)

A standard calibration curve was prepared using a standard method to obtain an equation (y = mx + c). 1 mL of each of the extract samples were transferred into a separate test tube. 5 mL of a 10% aqueous dilution of Folin-Ciocalteau reagent was added into the test tube [8]. The solution mixed using vortex mixer. After 3-8 minutes, 4 mL of aqueous sodium carbonate (7%) was added and mixed again thoroughly for another 1 minutes and incubated in water bath at 45 ºC for 15 minutes. All measurements are duplicated. Then, the absorbance value of the prepared solution taken using UV-VIS Spectrophotometer at 760nm. The absorbance value obtained from the sample was used together with the equation from standard calibration curve plotted to obtain the Total Phenolic Content in the sample expressed as Gallic acid equivalents (GAE mg/g).

2.6 Evaluation of Antibacterial Activity

The antibacterial activity of the herbs extracted at the most optimum condition was determined by the Kirby-Bauer disc diffusion method. Different nutrient agar plates ware inoculated with test organisms, which are *Escherichia coli* and *Staphylococcus epidermidis* and spread using glass spreader. Sterile paper discs with 6mm in diameter were impregnated in each herb extracts solution allowed to dry at room temperature for about 15 minutes. By using sterile forceps, the discs were placed on the inoculated agar surface in duplicates. The plates were incubated for 24h at 37°C [9]. A standard antibiotic discs of penicillin was used as positive control and another discs containing distilled water served as negative control. Antibacterial activity was determined by the diameter of clear inhibition zone around the discs.

3. Results

3.1 Effects of Extraction Method on Antioxidant Activity (AA) and Total Phenolic Content (TPC).

The antibacterial activity of the herbs extracted at the most optimum condition was determined by the Kirby-Bauer disc diffusion method. Different nutrient agar plates ware inoculated with test organisms, which are *E. coli* and *S. epidermidis* and spread using glass spreader. Sterile paper discs with 6mm in diameter were impregnated in each herb extracts solution allowed to dry at room temperature for about 15 minutes. By using sterile forceps, the discs were placed on the inoculated agar surface in duplicates. The plates were incubated for 24h at 37°C [9]. A standard antibiotic discs of penicillin was used as positive control and another discs containing distilled water served as negative control. Antibacterial activity was determined by the diameter of clear inhibition zone around the discs.

Table 1 - Comparison the values of AA and TPC between different extraction method

Extraction method	Antioxidant activity (% scavenging)		Total phenolic content (mg GAE/g)	
	Asystasia gangetica	*Phyllanthus niruri*	*Asystasia gangetica*	*Phyllanthus niruri*
Traditional	21.05 ± 4.03^{c}	27.45 ± 16.86^{b}	2.74 ± 0.01^{a}	2.61 ± 0.03^{a}
Soxhlet extraction	37.94 ± 5.33^{b}	21.56 ± 25.19^{b}	2.64 ± 0.01^{a}	2.73 ± 0.08^{b}
Cold maceration	59.57 ± 0.86^{a}	51.15 ± 4.95^{a}	2.75 ± 0.06^{a}	2.68 ± 0.08^{b}

[a,b,c] Mean values (means ± standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test with n=4 (Minitab v18, Statistical Software).

3.2 Evaluation of Cold Maceration, Ultrasonic Assisted Extraction (UAE) and Combined Cold Maceration – UAE at Optimum Condition.

The optimal UAE conditions are dependent on the type of herb [10]. RSM was used to optimize the UAE where this study reveals *P. niruri* consumed shorter extraction time than *A. gangetica* but higher power needed to extract the highest yield of antioxidant and phenolic compound (98.14W and 0.17mins and 17.57W and 5.83mins respectively). The efficient extraction techniques were compared between cold maceration, UAE and combined cold maceration-UAE. UAE produces a cavitation, which further leads in production, growth and collapse of bubble [11]. This result supports the previous study which state UAE do improve the efficiency of conventional extraction method [12] that indicate about double to triple increasing in yield of antioxidant activity for *A. gangetica* and *P. niruri*. Besides, combined cold maceration-UAE is a new approach to extract the antioxidant and phenolic compound in herbs. The result in this study shows combined extraction technique was successfully conducted which exhibit the highest yield of antioxidant and phenolic compound.

Table 2 - Comparison the values of AA and TPC between cold maceration, UAE and combined cold maceration-UAE

Extraction method	Antioxidant activity (% scavenging)		Total phenolic content (mg GAE/g)	
	Asystasia gangetica	*Phyllanthus niruri*	*Asystasia gangetica*	*Phyllanthus niruri*
Cold maceration	23.77 ± 0.04^{c}	33.88 ± 0.07^{c}	2.74 ± 0.01^{c}	2.86 ± 0.01^{b}
UAE	75.86 ± 0.00^{b}	63.64 ± 0.70^{b}	2.79 ± 0.00^{b}	2.27 ± 0.01^{c}
Combined cold maceration-UAE	84.15 ± 0.04^{a}	94.04 ± 0.00^{a}	3.47 ± 0.02^{a}	3.13 ± 0.00^{a}

[a,b,c] Mean values (means ± standard deviation) within the same column with different letters are significantly different (p) according to Tukey's HSD test with n=2 (Minitab v18, Statistical Software).

3.3 Evaluation of Antibacterial Potential of *Asystasia Gangetica* and *Phyllanthus Niruri* Extract.

Table 3 shows the result obtained of combined cold maceration-UAE at optimum condition for both *A. gangetica* and *P. niruri* were potent against the test organism. It was observed that the *A. gangetica* extract exhibited more potency than the *P. niruri* extract. The growth of *E. coli* and *S. epidermidis* were inhibited in both extracted herbs.

Table 3 - The antibacterial testing against *Escherichia coli* and *Staphylococcus epidermidis*

Test Bacteria	Inhibition zone (mm)	
	Asystasia gangetica	***Phyllanthus niruri***
Escherichia coli	11.50 ± 0.71	8.00 ± 0.00
Staphylococcus epidermidis	9.00 ± 1.41	6.75 ± 0.35

4. Conclusions

The study indicates the presence of antioxidant activity, total phenolic content and antibacterial properties in extracted herbs based on different extraction method. In summary, both *Asystasia gangetica* and *Phyllanthus niruri* extract have great potential as natural antioxidant sources, which also show the antibacterial properties. Hence, both herbs may be used as antibacterial agents.

Acknowledgement

Appreciation also goes to everyone involved directly or indirectly towards the compilation of this research.

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