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Short Communication

Phytochemical, Antioxidant and Antibacterial Evaluations of *Ipomoea batatas* L. from Riau, Sumatera Island, Indonesia

Dian Mayasari[1,3]*, Deri Islami[1], Eva Oktariani[2], Putri Wulandari[1]

[1]*Department of Pharmacy, Faculty of Pharmacy and Health Sciences, Universitas Abdurrab, Pekanbaru 28291, Indonesia*
[2]*Department of Medicine, Faculty of Medicine, Universitas Abdurrab, Pekanbaru 28291, Indonesia*
[3]*Indonesia Phytomedicine for Metabolic Diseases Centre, Universitas Abdurrab, Pekanbaru 28291, Indonesia.*

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ABSTRACT

Indonesia is rich in its biodiversity of medicinal plants used traditionally for healing several ailments. *Ipomoea batatas* L., one of the traditional herbs of Indonesia that is popularly known as sweet potato, has played a significant role as a source of energy and phytochemicals in human nutrition and animal feeding. In traditional use, *Ipomoea batatas* (*I. batatas*) is effectively utilized in herbal medicine as an anti-inflammatory agent for treatment of illnesses and infectious oral diseases. This study aimed to evaluate the phytochemical screening, with the antibacterial and antioxidant activity of extracts of *I. batatas* leaves. Antibacterial effect was tested against Gram-positive bacteria, Gram-negative bacteria and resistant bacteria through the agar disc diffusion method while antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method. The phytochemical screening showed the ethanol extract contains alkaloids, triterpenoids, flavonoids, tannins and saponins. The IC_{50} value for antioxidant properties was 233.476±0.01 ppm. Antimicrobial activity showed that percentage of inhibition growth was 83.61%. Ethanol extract showed the moderate antibacterial activity against Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus mutans* with diameter inhibition zone 8.7±0.01 cm and 10.2±0.3 cm in concentration 40%, respectively. Owing to the presence of its rich variety of secondary metabolites, the leaves extract of *Ipomoea batatas* L. is expected to exhibit more medicinal properties and be useful to develop as therapeutic agents in the future.

***Keywords*:** *Ipomoea batatas* L., antibacterial, traditional herbs, sweet potato, free radical scavenging.

Introduction

Antioxidants prevent oxidative stress-related illnesses and particularly preserve the ideal balance of life by boosting the body's endogenous immune systems against infectious disease.[1] Natural products have an abundance of antioxidant compounds, naturally occurring in plant sources, which are proven to be effective free radical scavengers.[2] The high content of the phenolic compounds plays a key role in their antioxidant activity regarding protection against the damage of reactive oxygen species. The phenolic compounds have been identified to be responsible for other biological effects, including antiviral, antibacterial and antitumor.[3]

In recent years, increasing incidence of microorganism resistance has forced scientists to discover new antibiotics. The discovery of antimicrobial agents from higher plants exhibit a promising source of antibiotics. The usage of plants as a source of anti-infective compounds has been implemented for many centuries. Traditional plants have been used for health over many generations by indigenous communities all around the world in the recovery from various types of ailments. The use of plants as alternative and complementary medicine is largely due to the belief in their safety and typically, they have little to no side effects.

*Corresponding author. E mail: dian.mayasari@univrab.ac.id
Tel: +628 2273 551812

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However, this needs to be investigated further to prove their efficacy and the safety of these traditional medicines.

The *Ipomoea* genus belongs to the Convolvulaceae family that includes more than 600 plant species distributed worldwide, which grow as weeds, or are cultivated as medicinal and ornamental plants.[4] One of the *Ipomoea* genus that is known worldwide is yellow sweet potato (*Ipomea batatas* Lam). *I. batatas* (L.), popularly known as the sweet potato (SP), has played a significant role as a source of energy and bioactive compounds in human nutrition and animal feeding. The different parts of this plant have been studied since they are used in folk medicine for the treatment of several disorders including acne and anemia, due to its antiviral applications, and agency as a anthelminthic vermifuge or dewormer.[5] In addition to their antidiabetic and antiviral properties, *I. batatas* leaves have been used to treat infectious disorders in Brazil.[6] Several studies on the leaves of *I. batatas* collected from Malaysia and Brazil have found excellent antioxidant activity due to the presence of flavonoids, *β*-carotene, anthocyanins and phenols.[7] Other pharmacological properties of *I. batatas* leaves are specifically as antiinflammatory, aphrodisiac, anticonvalescent, antitumor, antidiabetic, antiviral, antioxidant and anticancer.[8] The grounded leaves of the sweet potato have also been applied as treatment for body parts swollen from inflammation. Phytochemical screening can be used to identify the chemical compositions of plant material sources. Based on its phytochemical profile, there are many benefits of *I. batatas* leaves regarding its chemical substance.[6]

The tuberous root of *I. batatas* is a high-quality source of carbohydrates, dietary fiber, vitamin A, vitamin B6, vitamin C, manganese, copper, potassium, and iron. Recently, studies of *I. batatas* focused on its antioxidant capacities due to the high content of phenols, flavonoids, and its derivatives.[9] The leaves contain a significant amount of protein, showing high amino acid stores. The leaves also contain several essential vitamins such as carotene, vitamin B2, vitamin C and vitamin E.[10]

Phytochemical substances protect the body against degenerative disorders such as cancer and atherosclerosis caused by free radicals. Because of their potential antioxidant and antibacterial properties as bioactive chemicals, the use of *I. batatas* leaves has been considered for the management of several infectious diseases. Accordingly, this study aimed to identify the phytochemical profiling compounds of *I. batatas* leaves collected in the Riau province, Sumatera Island, Indonesia and to study their antioxidant and antibacterial activity against several pathogenic microorganisms.

Materials and Methods

Chemicals and reagents
All solvents: n-hexane, ethyl acetate, ethanol, formic acid, acetic acid, methanol, chloroform was of analytical grade supplied from Merck (Merck, Darmstadt, Germany). Phytochemical profiling regents: ferric (III) chloride, Mayer, Wagner, Dragendorff, Lieberman-Burchard reagent were used for phytochemical assay. Extracts were monitored by thin layer chromatography (TLC) silica gel 60 F_{254} nm plates (Merck). Chloramphenicol was used as positive controls and dimethyl sulfoxide was used for antibacterial testing. 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) assay was used to measure the free radical scavenging activity. All chemical solvents used were analytical grade.

Preparation of samples
The fresh mature leaves of *Ipomoea batatas* L. (Figure 1) were collected from the Tampan district, Pekanbaru, Riau province, Sumatera Island, Indonesia on June 2021. Plant specimens were authenticated at the Department of Biology, Faculty of Mathematics and Sciences, University of Riau, Indonesia. Voucher specimen number was (234/UN19.5.1.1.3-4.1) and deposited at the Faculty of Mathematics and Sciences, University of Riau. Leaves were washed and dried in hot air oven at 40°C for 48 hours and homogenized to a fine powder. The powders were stored in plastic packaging and sealed for further analysis.

Preparation extracts of I. batatas leaves
Two hundred and fifty grams of the leaf powder of *I. batatas* were macerated with 2,5 L ethanol 96%. The extract was filtered and the solvent was evaporated by a rotary evaporator vacuum to obtain a viscous semi-solid mass. The yield of the extract was calculated and recorded. This semi-dry crude ethanol extract was subjected to TLC analysis and continued to examination by phytochemical profiling and biological activity assay (antioxidant and antibacterial activity). Percentage of extract rendement was measured by the following formula:

$$\% \text{ extract rendement} = \frac{\text{weight crude extract (g)}}{\text{weight of sample (g)}} X\ 100\%$$

Thin layer chromatography (TLC)
TLC experiments were conducted in 10x20 cm TLC Silica 60 plates (Merck) with the mobile phases containing several steps including, ethyl acetate: formic acid: acetic acid: water (100: 11: 11: 26 v/v/v/v), n-butanol: glacial acetic acid: water (4: 1: 2 v/v/v), ethyl acetate: methanol: ammonia (8: 1.9: 0.1 v/v/v), and n-hexane: ethyl acetate (7: 3 v/v). All mobile phases were placed in a saturated chamber for 20 min., then after development, the plates were dried in a stream of warm air. Next, the plates were visualized at ultraviolet (UV) light wavelengths of 245 nm and UV 366 nm used for phytochemical screening by spraying several reagents.

Phytochemical screening of ethanol extract I. batatas leaves
Qualitative phytochemical screening (alkaloids, flavonoids, terpenoids, steroids, saponins and tannins) of ethanol extract of *I. batatas* L. leaves was done according to the standard qualitative analysis of phytogenic compounds described by Harborne *et al*.[12]

Antioxidant activity
The antioxidant activity of the extracts was determined by the free radical scavenging DPPH test. The analysis procedures were done following the steps described in our previous publication.[13] For DPPH testing, the results were expressed in IC_{50} (concentration of the extract that allows 50% of DPPH free radicals to be trapped) and were calculated using the exponential regression equation.

Antimicrobial assay
The antibacterial activity was examined using the disc diffusion agar method. The pathogenic microorganisms used in this study from American Type Culture Collection (ATCC) *Streptococcus mutans* (*S. mutans*) ATCC 25175 and *Staphylococcus aureus* (*S. aureus*) ATCC 25923 as Gram-positive bacteria, *Escherichia coli* (*E. coli*) ATCC 25922 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 9027 as Gram-negative bacteria and one resistant bacteria, *Methicillin-resistant Staphylococcus aureus* (MRSA) ATCC 33591. The procedures were conducted following the steps described in our previous publication with slight modification.[14] Antibacterial properties were measured by the minimum inhibitory concentration (MIC) value, which shows the extract concentration that allows 50% inhibition of the growth of microorganisms.

Statistical analysis
Analyses were done in triplicate and the data underwent testing with one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS version 16.0, IBM Corp., Armonk, NY, USA).

Figure 1: Sweet potato leaves (*Ipomoea batatas* L.)[11]

Results and Discussion

Extraction and TLC analysis
Extraction was done by the maceration method using ethanol solvent yielded rendement 19.821% (99.105 g). The ethanol extracts, whose composition was not known yet, were analyzed on silica gel layers with the aid of three solvent systems. The extracts were analyzed by TLC using various mobile phases: ethyl acetate: formic acid: acetic acid: water (100: 11: 11: 26 v/v/v/v), *n*-butanol: glacial acetic acid: water (4: 1: 2 v/v/v), ethyl acetate: methanol: ammonia (8: 1.9: 0.1 v/v/v), and n-hexane: ethyl acetate (7: 3 v/v) (Figure 2).
The TLC analysis of the crude extracts of the *I. batatas* leaves extract showed good separation. After separated from the ethanol extract of *I. batatas* leaves in a suitable composition solvent system, the mobilities of all compounds were recorded. Figure 2 shows the separation of the mixture compounds based on their Rf values. The TLC technique can separate the chemical constituents of a mixture sample to identify its chemical component.[15]
Three different images of the mobile phases on TLC plates (A, B, and C) with visible light showed yellow color with R_f values of 0.6-0.9 (A); 0.00-0.50 (B); and 0.28, 0.69, 0.79 (C). The UV 254 nm light showed black spots (R_f: 0.6-0.9 (A); 0.00-0.50 (B); and 0.00-0.20 (C) on green background that means they have several compounds on it. Different from visible light and UV light at 245 nm, UV light at 365 nm identified various fluorescent light color spots (R_f: 0.69, 0.75, 0.81, 0.94 (A); 0.19, 0.30, 0.49, 0.73, 0.86, 0.93 (B); and 0.06, 0.11, 0.14, 0.23, 0.26, 0.35, 0.56, 0.68 (C) on the TLC plates with blue light background. The Rf values, color on TLC plates, size and shape of detection zones under visible, 245 nm and 365 nm UV light were evidence for identification of the characteristics of the samples.[16]

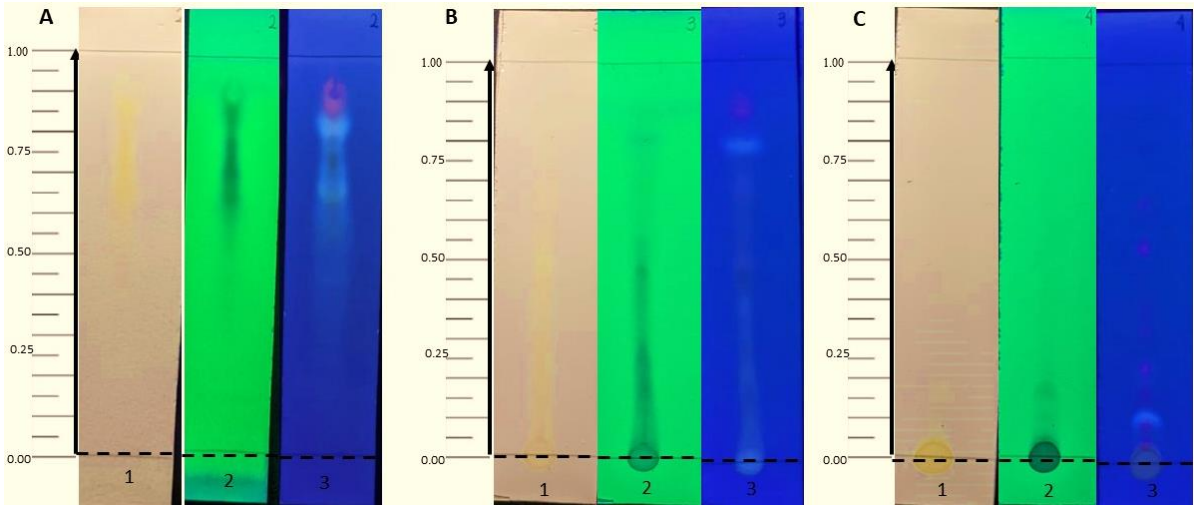

Figure 2: TLC profiles of ethanol extract using several mobile phases: *n*-butanol: glacial acetic acid: water (4: 1: 2 v/v/v) (A), ethyl acetate: methanol: ammonia (8: 1.9: 0.1 v/v/v) (B), *n*-hexane: ethyl acetate (7: 3 v/v) (C) with visible light (1), UV light wavelength 254 nm (2) and 365 nm (3).

TLC is one of the most common and simplest of the analytical methods used to achieve the compound identification purposes, and it has many valuable analytical applications in the fields of food analysis and quality control.[17] Identification of flavonoid compounds is usually performed under UV light at 254 nm (all flavonoids cause fluorescence quenching) or at 365 nm (depending on the structural type showing dark yellow, green or blue fluorescence). Fluorescence can be enhanced through several spray reagents, which will lower the detection limit.[18] The orange-yellow is indicative of the presence of flavonol glycosides. The vivid yellow-green colour might be due to the presence of flavone glycoside bioflavonols and unusually substituted flavones. The blue bands could be due to presence of 5-deoxyisoflavones and 7,8-dihydroxy-flavanones. Furthermore, the blue bands could be due to the presence of anthocyanidins-3,5-diglycosides. [19,20]

The phytochemical screening for major secondary metabolites can be investigated by color detection and the change of colour solution of the test samples. The results of the qualitative analysis test performed on ethanol extracts from *I. batatas* leaves are represented in Table 1.

Findings from the qualitative phytochemical analysis of *I. batatas* leaves extract showed the presence of alkaloids, flavonoids, tannins, triterpenoids and saponins and absence of steroids. Secondary metabolites of the plants constitute biologically potent compounds which are being harnessed as pharmacological agents.[21] These compounds are identified through phytochemical screening of secondary metabolites: alkaloid testing through Mayer, Wagner and Dragendorff reagents resulting in white, brown, and red orange precipitate, respectively, flavonoid testing resulting in orange color, triterpenoids forming brown rings, steroids resulting in bluish green color, tannins resulting in dark green color solution and saponins forming stable foam.

These findings are similar to the results published by Pochapski *et al*.[6] Phytochemical profiling was purposed to explain the group of secondary metabolites that form in the plants. Alkaloid compounds have been related to antiviral, antispasmodic, anti-Parkinson's, antihypertensive agent, antitussive and analgesic agency.[22] Flavonoids possess antioxidant, antithrombotic, antibacterial, antiviral, anti-inflammatory effects and reduce oxidative stress related to cardiovascular disorders.[23] Triterpenoids have been identified to have a variety of biological activities including antioxidant, antimicrobial, antiviral, antiallergic, antiangiogenic, anticancer and spasmolytic activity.[24] Tannins have been shown to have antioxidant properties, anticarcinogenic, antimutagenic potentials and antimicrobial agency.[25] Saponins consist of an aglycone unit linked to one or more carbohydrate chains. Saponins have exhibited an abundance of pharmacological activities including antiviral, anticancer, anti-inflammatory, antifungal, antimicrobial, antioxidant and immunomodulatory effects.[26]

Antioxidant activity

Ipomoea batatas has been known to possess a high content of anthocyanins which contribute to its antioxidant activity. The antioxidant activity of the ethanol extract of *I. batatas* leaves is expressed in IC_{50}. The ability of the sample to trap free radicals was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test. The DPPH method is a rapid and simple method to investigate antioxidant capacity. The DPPH free radical scavenging method offers the primary approach for investigating the antioxidant potential of a compound, an extract or other biological sources. This is the simplest technique, wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period. The radical scavenging activity is determined from a reduction in the absorbance at 517 nm and reduction of the violet color to yellow color.[27] Table 2 shows the results of antioxidant properties of ethanol extract of *I. batatas* leaves through the DPPH free radical scavenging method.

The results show that the percentage of inhibition varies linearly with the concentrations from 20% to 80% (b/v). The results in the linear range were statistically analyzed in order to determine the IC_{50} values for the samples and standard. The IC_{50} values were used to reflect the antiradical scavenging ability. The meaning of IC_{50} value is the capability of an antioxidant and necessary concentration to achieve 50% of the scavenging DPPH free radical capability. This means the smaller the IC_{50} value is, then the higher is the free radical scavenging activity, and vice versa.[20] The IC_{50} values were concentration dependent and varied depending on the samples. Antioxidant activity of the ethanol extract showed moderate capacity. This finding is based on a previous study that indicated the antioxidant activity could be divided into five groups according to their IC_{50} values such as highly active (<50 µg/mL), active (50-100 µg/mL), moderate (101-250 µg/mL), weak (250-500 µg/mL) and inactive (>500 µg/mL).[28]

The DPPH assay used in this study revealed that the sample possessed scavenging action on active radicals by donating hydrogen atoms to DPPH molecules. The scavenging activity of DPPH radicals was estimated to happen since there is hydroxyl group in the components of ethanol extract which can donate its electron to DPPH radical. The DPPH assay is largely used for the assessment of drugs with antioxidant potential.

A previous study regarding antioxidant properties of *I. batatas* leaves from Cameroon revealed that the total antioxidant capacity was in the range of 19.00 to 23.48 mg AAE/gDW, while IC_{50} ranged from 1.58 mg/mL to 3.08 mg/mL. In addition to this, they also investigated the anthocyanin content and found proanthocyanidins content in the range from 175.27 g GAE/100 gDW to 176.04 mgCE/100 gDW.[5] Flavonoids act as an antioxidant with radical scavenger activity. The mechanisms of antioxidant action of flavonoids include suppressing reactive oxygen

formation, either by inhibition of enzymes or chelating trace elements, scavenging reactive oxygen species and upregulating antioxidant defenses.[29] According to Khoddami *at al*[30] in 2013, the suppression properties of antioxidants might be caused by the glycosylation of the 3-OH and at positions 4' and 7'-OH.[30]

Antibacterial activity

The antibacterial activity of the extracts of *I. batatas* L. leaves was measured by the agar disc diffusion method. This method was observed using sterile paper as an absorption media of the sample and the clear zone around the paper was recorded. The dried extract of *I. batatas* leaves was dissolved in an aqueous solution of 50% (v/v) dimethyl sulfoxide (DMSO) in order to obtain 20%, 40%, 60%, and 80% sample solutions.

The antibacterial activities of *I. batatas* leaf extracts are shown in Table 3. The susceptibility of each test microorganism to the plant extracts was shown by clear zones of growth inhibition, thus showing the relative activity of the test plant extracts against the microorganisms. The highest zone of inhibition was from the highest concentration of plant extract (80% b/v).

The ethanol extract showed the highest zone of inhibition at 80% (b/v) against *S. mutans* and *S. aureus* as Gram-positive bacteria, *E. coli* and *P. aeruginosa* as Gram-negative bacteria and MRSA as resistant bacteria. The results showed that the antibacterial activity of ethanol extract of *I. batatas* leaves against the Gram-positive bacteria was more significant than that against the Gram-negative bacteria. This finding could be due to the presence of the hydrophilic surface of the Gram-negative outer membrane, consisting of lipopolysaccharide compounds that provide a barrier to penetration as well as enzymes in the periplasmic space that are capable of the breaking down any unknown molecules from the outside. The presence of the outer membrane permeability barrier of Gram-negative bacteria can limit the access of the antimicrobial agents to the target in the bacterial cell. This result can explain why Gram-positive bacteria are more sensitive and give more significant result on antibacterial tests than Gram-negative bacteria. The similar finding is also published in the previous study conducted by Mayasari *et al*. in 2021.[13]

The antimicrobial activity of a sample is due to the presence of secondary metabolites responsible for reducing bacterial growth. This was supported by previous research that found phenolic hydroxyl groups in flavonoid compounds are able to bind to bacteria with the presence of 4'-hydroxyl groups in the B ring and 5,7'-dihydroxyl in the A ring.[31] Although the extract showed low antibacterial activity, since the single purified compounds have the possibility of antibacterial activity, then the more in-depth discovery of active compounds with antibacterial agency needs to be done in the future. This result implied that *I. batatas* can be considered an important candidate plant to isolate and characterize further natural products based on its bioactive molecules.

Table 1: Phytochemical screening of *I. batatas* leaves ethanol extract

No.	Phytochemical test	Result
1.	Alkaloids	+
2.	Flavonoids	+
3.	Triterpenoids	+
4.	Steroids	-
5.	Tannins	+
6.	Saponins	+

Table 2: Effect of ethanol extract of *I. batatas* leaves on DPPH free radical scavenging method

Concentration (µg/ml)	Ln concentration	Absorption sample	% inhibition	IC_{50} (µg/mL)
1000	6.908	0.175 ± 0.014	84.35 ± 0.01	
500	6.215	0.178 ± 0.005	83.61 ± 0.05	
250	5.521	0.221 ± 0.01	45.47 ± 0.03	233.476 ± 0.01
125	4.828	0.418 ± 0.01	24.22 ± 0.01	
62.5	4.135	0.466 ± 0.01	12.27 ± 0.01	
31.25	3.442	0.486 ± 0.004	7.414 ± 0.08	

n=3, result expressed as Mean ± Standard Deviation.

Table 3: Antibacterial activity of ethanol extract of *I. batatas* leaves using agar disc diffusion method

Microorganisms	Concentration (%)			
	20	40	60	80
Gram-positive				
Streptococcus mutans	9.0 ± 0.2	10.2 ± 0.3	11.4 ± 0.1	12.2 ± 0.2
Staphylococcus aureus	8.5 ± 0.3	8.7 ± 0.01	9 ± 0.01	10.75 ± 0.5
Gram-negative				
Escherichia coli	7 ± 0.02	8.2 ± 0.2	8.4 ± 0.9	8.6 ± 0.3
Pseudomonas aeruginosa	7.8 ± 0.4	8.5 ± 0.4	8.6 ± 0.7	8.9 ± 0.9
Resistant-bacteria				
MRSA	7.7 ± 0.9	8.0 ± 0.3	8.2 ± 0.3	8.25 ± 0.02
Positive control				
Chloramphenicol	18.50 ± 0.02	18.75 ± 0.6	19.3 ± 0.2	19.8 ± 0.01

n=3, result expressed mean of diameter zone inhibition (cm) ± Standard Deviation. MRSA: Methicillin-resistant *Staphylococcus aureus*.

Conclusion

The phytochemical analysis indicated the leaf extract of *I. batatas* contains alkaloids, flavonoids, terpenoids, tannins and saponins. *I. batatas* L. leaves have potential anti-oxidant activity through DPPH free radical scavenging pathway. However, further investigations are needed to determine the bioactive compound compositions, pharmacological applications and toxicity of these extracts. Thus, in the future, *Ipomoea batatas* L. might be a potential source of natural antioxidants and antiinfectious agents from Riau, Indonesia.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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