Antifungal and antioxidant activities of the extract of *Stephania pierrei* tubers

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Abstract

Stephania pierrei (Menispermaceae) was climbers with membranous and peltate leaves and especially distributed in the East of Thailand. The plant tubers had been used for the treatment of body migraine, heart disease, and oedema. In this work, *S. pierrei* tubers were collected and prepared into the powder. The powder was extracted with water, evaporated, and freeze-dried for analysis of biological activities. The tuber extract of *S. pierrei* was tested the *in vitro* antifungal activity against three fungi including *Aspergillus niger*, *A. flavus*, and *Alternaria alternata*, and also studied antioxidant activity using 2, 2'-azino-bis (3-ethyl benzthiazoline-6-sulfonic acid) (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The result showed that moisture, protein, fat, ash, carbohydrate, and starch contents of the tuber powder were 12.20, 8.01, 0.50, 11.22, 68.07, and 37.53% (w/w), respectively. All of the tested strains were not inhibited by the extract at a concentration of 6 mg/mL. *Alternaria alternata* was more sensitive to the extract at a concentration of 12 mg/mL than the other strains. The IC_{50} values determined based on ABTS, DPPH, and FRAP (293 µg/mL, 412 µg/mL, and 0.17 µmol Fe^{2+}/mg dried extract, respectively) assays were low and showed the antioxidant activity.

***Keywords*:** *Proximate analysis, antioxidant activity, antifungal activity, aqueous extract, stephania pierrei, alternaria altermata*

1. Introduction

Nowadays, an increase in the incidence of fungal infections is due to the pathogens resistant to multiple antifungal agents. The antifungals comprise a large and diverse group of drugs used to treat fungal infections. The increased usage of antifungal agents in recent years has resulted in the development of resistance to drugs. In fact, the resistance to antibiotics during prolonged treatment with several antifungal drugs has been the reason for an extended search for newer drugs to treat fungal infections (Kalidindi et al., 2015). Several plant extracts have been used traditionally as antifungal agents against human, animal and plant pathogens (Barhouchi, Aouadi, & Abdi, 2018). Approximately 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system such as treating cancer, invasive aspergillosis and harmful diseases (Altemimi et al., 2017; Lan et al., 2018). Not only fungal pathogens but also free radicals could be a cause of diseases in human. Free radicals are fundamentals to biochemical process, represent an essential part of aerobic life and metabolism, and link to oxidative stress resulting in many diseases. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules and may protect cells from damage caused by unstable molecules known as free radicals. Low level of antioxidants has shown to be associated with many disorders including cancer, inflammation, coronary heart disease and diabetes (Rashed, 2014). Many plants have been also found to possess free radical scavenging activity (polyphenols, alkaloids, and terpenoids).

Researchers have interested to search for new bioactive compounds from plants for new antioxidant and antifungal agents. Natural products have been a source of traditional herbal medicine for the treatment of human diseases. Drugs from the plants are easily available, less expansive, safe and efficient, and rarely have side effects (Yadev & Agarwala, 2011). The plants genus *Stephania* distributed in tropical and subtropical Asia, tropical Africa, and Oceania (Dary et al., 2015). These plants had been used in folk medicine for the treatment of various ailments. Alkaloids, flavonoids, lignans, steroids, terpenoids, and coumarins had been identified and evaluated for biological activity (Semwal et al., 2010). Many researchers searched for biological activity from the extract of the genus *Stephania* sp. such as *S. cepharantha* (Rogosnitzky & Danks, 2011), *S. epigaea* (Lv et al., 2013), *S. glabra* (Semwal & Rawat, 2009; Semwal et al., 2012), *S. pierrei* (Kamonwannasit & Kamcharoen, 2018), *S. rotunda* (Baghdikian et al., 2013; Gulcin et al., 2010), *S. sinica* (Xie et al., 2014), *S. venosa* (Bunluepuech & Tewtrakul, 2011), and *S. yunnanensis* (Shi et al., 2015). *Stephania pierrei* Diels was a species recorded from the Indo-Chinese Peninsula. The tuber is

traditionally used in Cambodia for the treatment of body oedema, migraine, and heart disease. Its tuber extract possesses antibacterial (Kamonwannasit & Kamcharoen, 2018), antimalarial, and anticancer activities (Semwal et al., 2010). Nevertheless, the antioxidant activity and chemical compositions in this plant were not reported.

2. Objectives

The objective of this study was to determine the nutritional value, antifungal, and antioxidant activities of the extracts obtained from the tubers of *S. pierrei*.

3. Materials and Methods

3.1 Plant Material

The tubers were washed 3-4 times with tap water and cut into small pieces. The plant material was dried at 50 □C in a hot air oven and ground into the powder. *S. pierrei* extract was prepared by boiling 50 g of dried plant powder in 500 mL of distilled water for 30 min. The aqueous extract of *S. pierrei* was filtered through cotton gauze and centrifuged at 2,500 g for 10 min. The supernatant was collected and concentrated at 40 □C using a rotary evaporator under reduced pressure. The residue was freeze-dried in a lyophilizer.

3.2 Proximate analysis of the tubers of *S. pierrei*

The chemical compositions of the tuber powder of *S. pierrei* consisting of moisture content, crude protein, crude fat, and ash were analyzed according to the standard methods of Association of Official Analytical Chemists (AOAC, 2016). Carbohydrate was calculated by subtracting the sum of moisture, protein, fat, and ash from 100. Protein content was determined by the Kjeldahl method with a conversion factor of 6.25. Fat content was determined by extraction with petroleum ether using a Soxhlet apparatus. Ash content was determined by incinerating the sample in a muffle furnace at 600°C for 3 hr. Starch was measured by the polarimetric method.

3.3 Antioxidant activities

ABTS radical cation scavenging activity assay

The ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) assay method was carried out according to Re et al. (1999). Briefly, ABTS radical cation ($ABTS^{\bullet+}$) was produced by the reaction between 5 mL of 14 mM ABTS and 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$). The mixture was kept in the dark to react for 16 hr at room temperature so that it reached a stable oxidative state. The working solution was prepared by diluting the mixture with ethanol to achieve the absorbance of 0.700±0.020 at 734 nm. The extract (50 µL) at various concentrations was added to 950 µL of $ABTS^{\bullet+}$ solution and mixed well. The reaction mixture was performed at room temperature for 6 min. The absorbance was measured at 734 nm comparing to the butylated hydroxyl toluene (BHT) as standard.

DPPH radical scavenging activity assay

The free radical scavenging capacity of the extract was measured using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay method described by Bor et al. (2006). The extract of 100 µL with various concentration (0-50 µg/mL) was added to 4.0 mL of 50 µM DPPH in methanol solution, and the final volume was adjusted to 5.0 mL with deionized water. The mixture was incubated in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured with a spectrophotometer at 517 nm. Antioxidant activity was expressed as IC_{50}, which was defined as the concentration of the extract required to inhibit the formation of DPPH radicals by 50%.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was carried out by the method as described by Dordevic et al. (2010). The FRAP reagent consists of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3$ in the ratio of 10:1:1 (v/v). The extract of 50 µL was added to 1.5 mL of FRAP reagent, which was freshly prepared and warmed to 37°C before use. The mixture was measured its absorbance at 593 nm using a UV spectrophotometer after 4 min of incubation. The ferrous sulfate ($FeSO_4$) was used as a standard solution. The FRAP results were expressed as µmol Fe^{2+}/mg dry extract.

3.4 Antifungal activities

Antifungal activities of the extract were tested against three fungi including *Alternaria alternata* TISTR 3435, *Aspergillus niger* TISTR 3012 and *A. flavus* TISTR 3135. The strains were obtained from the Thailand Institute of Scientific and Technological Research.

The antifungal activity of the extracts against *A. alternata*, *A. niger* and *A. flavus* was determined by using agar well diffusion method (Thangavelu et al. 2013). The fungi suspensions were $6x10^4$ spores/mL in sterilized saline solution (0.85%) and 100 µL of the fungal suspension was spread onto the surface of the potato dextrose agar (PDA) in Petri dishes. Holes with diameters of 5 mm were punched with a sterilized cork borer. The extracts were added to each well with different concentrations (6, 12 and 24 mg/well). Subsequently, the plates were incubated at 30 □C for 72 hr. The diameters of the inhibition zones were measured in millimeters and all tests were performed in duplicate.

4. Results and Discussion

The proximate analysis showed the percentage of moisture content, crude protein, crude fat, ash, carbohydrate and starch of *S. pierrei* tuber powder as 12.20, 8.01, 0.50, 11.22, 68.07 and 37.53% (w/w), respectively (Table.1).

Table 1 Chemical compositions of powder of *S. pierrei* tubers

Compositions	**% (w/w)**
Moisture content	12.20±0.52
Crude protein	8.01±0.11
Crude fat	0.5±0.05
Ash	11.22±0.21
Carbohydrate	68.07±0.67
Starch	37.53±0.16

Ash content is an index of mineral content in the sample. In this study, ash content of 11.22% in *S. pierrei* tubers was measured in the sample, which was higher than other plants in Menispermaceae family (3.26-9.61%) such as *Tinospora cordifolia* stem (Chavan et al., 2014; Mahima et al., 2014), *T. malabarica* stem (Hedge et al., 2015), *T. sinensis* stem (Chavan et al., 2014), *Sphenocentrum jollyanum* root (Ekpone et al., 2018). However, the fat content of *S. pierrei* tubers (0.5%) was lower than other plants in this family. The protein content of *S. pierrei* tubers (8.01%) was closed to *T. cordifolia* stem (7.74%) (Mahima et al., 2014) and *S. jollyanum* root (7.35%) (Ekpone et al., 2018). The carbohydrate content of *S. pierrei* tubers 68.07% was slightly lower than *Sphenocentrum jollyanum* root (77.26%) (Ekpone et al., 2018), and was higher than *Tinospora* sp. (Chavan et al., 2014).

The aqueous extract of the tubers of *S. pierrei* was assessed for antifungal activity using agar well diffusion method by measuring the diameter of growth inhibition zones at different concentrations. The antifungal activity is presented in Table 2. The inhibition zone of *A. alternata* was 11.75±1.5 mm, whereas *A. niger* and *A. flavus* were not inhibited by the extract at concentration 12 mg/well. *A. alternata* (17.75±1.70 mm) was more sensitive to the extract than *A. flavus* (12.5±0.7 mm) at concentration 24 mg/well and *A. niger* (8.67±0.52 mm) at the same concentration of the extract. Semwal et al. (2009) reported that the tubers of *Stephania glabra* were found to be inhibitory for *A. niger*, *A. fumigatus*, *P. citranum*, *M. gypseum*, *M. canis*, and *T. rubrum*. Plants that are a rich source of bioactive compounds such as tannins, terpenoids, saponins, alkaloids, and flavonoids are reported to have *in vitro* antifungal properties (Arif et al., 2009). Consistent with Kamonwannasit and Kamcharoen (2018) reported that *S. pierrei* tuber contains alkaloids and tannins. However, the antifungal activity of the extract depends on the concentration of extract and strains of fungi.

Table 2 Antifungal activity (inhibition zone) of *S. pierrei* tuber extract at various concentrations

Fungal strains	Inhibition zone in diameter (mm)		
	Concentration of the extract		
	6	12	24
Aspergillus niger	ND	ND	8.67±1.52
Aspergillus flavus	ND	ND	12.5±0.70
Alternaria alternata	ND	11.75±1.5	17.75±1.70

ND: not detected; Values are expressed as means ± standard deviation (n=3)

Antioxidant activity of the aqueous extract of *S. pierrei* tubers was studied *in vitro* in three different radical models (ABTS, DPPH, and FRAP). The scavenging effects of the extract and standard (BHT and ascorbic acid) on the ABTS and DPPH radicals, respectively, were expressed as half maximum inhibitory concentration (IC_{50}) value. ABTS, DPPH, and FRAP assays are widely used to determine the antioxidant capacity in plant extracts because of their simplicity, stability, and accuracy (Reddy et al. 2010). The antioxidant activity of the extract was determined as seen in Table 3.

Table 3 Antioxidant activity of *S. pierrei* tuber extract

Methods	Standards		Aqueous extract
ABTS assay (IC_{50} µg/mL)	BHT	108.12±8.41	293.68±11.42
DPPH assay (IC_{50} µg/mL)	Ascorbic acid	1.86±0.12	412.09±19.40
FRAP assay (µmol Fe^{2+}/mg extract)	-	-	0.17±0.02

The IC_{50} value of BHT and the extract were 108.12 µg/mL and 293.68 µg/mL, respectively. The extract could seem to be slow and low efficient scavengers of the $ABTS^{\bullet+}$ radical. In DPPH assay, the antioxidant activity was obtained from ascorbic acid and the extract with IC_{50} values of 1.86 µg/mL and 412.09 µg/mL, respectively. The FRAP assay was used to evaluate the antioxidant properties of the extract based on their ability to reduce ferric (III) to ferrous (II) (Akter et al. 2016). The antioxidant activity of 0.17 µmol Fe^{2+}/mg extract was obtained from the FRAP assay. The result of ABTS, DPPH and FRAP assays showed that the extract was low effective on antioxidant activity. This result might be due to the differences in solvent extraction leading to low antioxidant activity. In addition, Naczk and Shahidi (2006) suggested that the polarity of the solvent illustrated a significant role in increasing the solubility of phenolic compounds. Srikong et al. (2017) studied the differential solvent extractions such as methanol, ethanol, dichloromethane, and hexane on antioxidant activity of the green seaweed *Ulva intestinalis*. They found that dichloromethane extract of *U. intestinalis* showed the highest antioxidant activity of the DPPH radical followed by the ethanol, methanol, and hexane, respectively. Pratap et al. (2013) reported that the aqueous extract exhibited strong DPPH radical scavenging ability (IC_{50} value of 82.05 µg/mL) than the methanolic extract (IC_{50} value of 987.51 µg/mL).

5. Conclusion

The results showed that the tubers of *S. pierrei* contained a moderate amount of protein, fat, ash, carbohydrate cellulose, hemicellulose, and starch. The extract of *S. pierrei* tubers has been shown to possess antifungal and antioxidant activities, which promoted by health benefits. Thus, it can be concluded that the presence of antifungal and antioxidant activities in the aqueous extract from *S. pierrei* tubers suggests that the plant may be a source of bioactive compounds with multi-bioactivity and material for functional food development.

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7. References

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