Bioactive Compounds and Antioxidant Activity of Leaves from *Piper sarmentosum* Piperaceae

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Abstract. *Piper sarmentosum* belongs to *Piperaceae* family, which is used as a cuisine in many Southeast Asian. This is a valuable herbal plant used as a traditional treatment for headaches, coughs, asthma, fever and as a spice in everyday cooking. The plant plays an important role in the antioxidant application. This study was conducted to analyze qualitative phytochemicals and assess the free radical potential, total polyphenol and flavonoid content of *Piper sarmentosum* leaves extracts. Using the standard procedure, the crude extracts were proved having lots phytochemicals such as alkaloids, coumarin, flavonoids, reducing compounds, saponins, tannins and terpenoids. The ethanolic extract from leaves of *Piper sarmentosum* had a medium total polyphenols content (60.61 ± 0.96 µgGAE/mg) and flavonoid content (70.14 ± 0.38 µgQE/mg). DPPH and ABTS radical scavenging activity increased proportionally with the concentration of *Piper sarmentosum*. IC₅₀ value of ethanolic extract were 264.20 ± 17.90 µg/mL (DPPH) and 176.40 ± 8.62 µg/mL (ABTS), respectively. The leaves of *Piper sarmentosum* can be used in the battle against diseases induced by free radicals such as asthma, diabetes mellitus, and coronary artery disease as a natural supply of antioxidants.

1. Introduction

Plants can synthesize a wide range of chemical compounds that have beneficial effects when used for the treatment of human diseases [1-4]. *Piper sarmentosum* Piperaceae (*P. sarmentosum*) is commonly distributed in Cambodia, Lao, Thailand, Indonesia, Vietnam and China. There are many records regarding the common uses of different parts of *P. sarmentosum*. In Thailand, the leaves were traditionally used as a carminative and the whole plant was used as an expectorant, antispasmodic and antiflatulence, and to relieve muscle soreness, asthma and cough. *P. sarmentosum* has been commonly used in the traditional Malaysian medicine to cure diabetes mellitus, joint aches and diseases of the gum hypertension [5]. In recent years, there have been many studies explaining the effects and mechanisms on *P. sarmentosum* treating various diseases. For example, the plant stimulated the treatment of endothelial dysfunction and atherosclerosis by suppressing TNF-α-induced VCAM-1 and ICAM-1 expression of NF-αB signaling and modulated the DDAH-ADMA pathway in human cultured
endothelial vein cells exposed to TNF-α [6]. Moreover, Asaricin and isoasarone inhibitory ability in *P. sarmentosum* can help the therapeutic uses of these compounds in breast cancer [7]. *P. sarmentosum* leaves aqueous extract attenuated the endothelial vascular dysfunction in spontaneously hypertensive rats [8].

*P. sarmentosum* is well-known for its strong amounts of antioxidants. According to Mohd et al. (2015), its antioxidant activity may reduce oxidative stress damage, increase NO output and reduce blood pressure and cholesterol rates [8]. The antioxidant function of *P. sarmentosum*’s methanol extract was proposed to derive from the presence of naringenin, which is a natural, active antioxidant compound [10]. *P. sarmentosum* has a significant defensive role as omeprazole against stress-induced gastric lesions. The protective effect was associated with reduced lipid peroxidation, increased prostaglandin E2, reduced gastric acidity, and reduced expression of COX-2 mRNA, which has been altered by stress [10]. Its antioxidant function not only occurs in the pharmaceutical industry but also allows the use of antibiotics in livestock feed as a potential substitute.

However, the phytochemical profile of *P. sarmentosum*, as well as the total content of phenolics and flavonoids remain little-known. Therefore, the present study was conducted to examine qualitative phytochemical and determine the free radical capacity, total polyphenol content, and flavonoid content of ethanol and aqueous extracts from *P. sarmentosum* leaves, owing to its many valuable uses.

2. Materials and methods

2.1. Materials and chemicals
Fresh plants of *P. sarmentosum* were purchased from local markets in Ho Chi Minh City, Vietnam during January–February 2020. Leaves of *P. sarmentosum* have been washed with tap water. It was then dried in a hot air-blowing oven at 50 °C until it had less than 15% water content. The leaves were grounded into fine powder and extracted using ethanol 96% and water, forming ethanol and aqueous extracts of *P. sarmentosum*. The ethanolic extract was used for the analysis of total polyphenols, total flavonoids compounds and antioxidant activity. All chemicals and solvents used in this study, including gallic acid, quercetin, vitamin C and ethanol were of analytical grade.

2.2. Evaluation of chemical components
Different chemical reactions such as methanol, diethyl alcohol, ammonia, sodium hydroxide, potassium chloride, hydrochloride acid, gallic acid, ferric chloride, aluminum chloride, quercetin and L-ascorbic acid were used for the identification of compound groups in each sample.

2.3. Total polyphenols contents (TPC) determination
The TPC values of *P. sarmentosum* ethanol and aqueous extracts were measured using a modified Folin-Ciocalteu method [12-14]. Each extract was combined with Folin-Ciocalteu reagent 10% (v/v). After 5 min, the solvent was added with Na₂CO₃ 20% (w/v), then shaken vigorously and incubated in the dark for 60 min. Optical absorbance was measured at 765 nm. The gallic acid used as a base for evaluating the total amount of polyphenols (µgGAE/mg dry weight of the sample).

2.4. Total flavonoids contents (TFC) determination
The total content of the flavonoids of *P. sarmentosum* ethanol and aqueous extracts was calculated by colorimetric aluminum chloride process, according to Ebrahimzadeh et al (2018) [15;16]. First, the extracts was mixed with AlCl₃ 10% and CH₃COOK 1M and vigorously shaken. After that the solvent was adjusted with distilled water to a volume of 5 ml. The mixture was incubated for 30 minutes in the dark. The absorbance was measured at 415 nm [17-19]. The total flavonoids content results were shown in µg of quercetin equivalents per dry weight of the sample (µgQE/mg).

2.5. The antioxidant activity
The antioxidant activity of *P. sarmentosum* ethanol and aqueous extracts was tested by DPPH and ABTS free radical scavenging tests as described by Islam et al. (2019) [20-22]. The working solution of reagent
was prepared to have absorbance value of 1.1 ± 0.02. Optical measurement of mixture at 517 and 734 nm by UV / VIS-1800 Spectrometer. After pre-concentration, 1 mL sample was mixed with 3 mL working solution of reagent. The solution was incubated in the dark within 30 min at room temperature. Vitamin C was used as the positive reference. The percentage of DPPH scavenging impact was determined using the following equation (1):

DPPH or ABTS scavenging effect (%) or inhibition percentage (I%) = \frac{Ab - As}{Ab} \times 100  \tag{1}

**Abbreviations:** Ab - Blank sample absorbance. As - Absorbing material, %I - inhibition percentage. The IC\(_{50}\) value was the sample concentration that inhibited the percentage to 50%. IC\(_{50}\) values are thus negatively associated with the antioxidant activity, the lower IC\(_{50}\) value indicates the maximum antioxidant activity of the sample being tested.

2.6. Statistical analysis

All experiments were conducted in three replicates and data was represented as mean ± standard deviations (SD). One way Variance Analysis (ANOVA) was used to compare the mean values. \(p\) values below 0.05 were considered as significant difference. Differences among the samples were measured using Tukey’s SPSS test. (version 23, IBM, USA) [23].

3. Results and Discussion

3.1. Evaluation of chemical components

The findings of our preliminary phytochemical study of extracts from *P. sarmentosum* leaves were reported in Table 1, which identified the presence of eight compounds such as alkaloids, antraquinons, coumarin, flavonoids, reducing sugar, saponins, tannins and terpenoids. Our results were similar to Seyyedan et al. (2013) that *P. sarmentosum* is a potential herbal plant with various compounds [25].

| Compounds              | Ethanolic extract | Aqueous extract |
|------------------------|-------------------|-----------------|
| Alkaloids              | +                 | -               |
| Antraquinons           | -                 | -               |
| Coumarins              | +                 | +               |
| Flavonoids             | +                 | +               |
| Reducing compounds     | -                 | +               |
| Saponin                | -                 | +               |
| Tannins                | +                 | +               |
| Triterpenoids          | +                 | -               |

Note: (-): Absent; (+): Present

3.2. Total polyphenols (TPC) and flavonoid content (TFC)

The ethanolic extract from leaves of *P. sarmentosum* showed a higher total polyphenols content (60.61 ± 0.96 μgGAE/mg) and flavonoid content (70.14 ± 0.38 μgQE/mg) than those of aqueous extracts (TPC and TFC values of 11.77 ± 0.43 μgGAE/mg and 29.1 ± 0.24 μgQE/mg, respectively). In research of Lee et al. (2011), total polyphenols contents was 50.01 mg GAE/g dry weight [25]. Previous study concluded that *P. sarmentosum* has antioxidant effect and contributes to antioxidant activity by its bioactive compounds such as polyphenols and flavonoids.
3.3. The antioxidant activity
Reactive oxygen species (ROS) build an extremely reactive state that can destroy other biomolecules, contributing to multiple chronic diseases such as diabetes mellitus, atherosclerosis, inflammation and cancers. *P. sarmentosum* is a herb with antioxidant activity and anti-atherosclerotic. The IC$_{50}$ value was the sample concentration that inhibited the percentage to 50%. IC$_{50}$ values are thus negatively associated with the antioxidant activity, the lower IC$_{50}$ value indicates the maximum antioxidant activity of the sample being tested. As shown in Figure 2, the DPPH and ABTS radical scavenging activity of *P. sarmentosum* ethanol extract was significantly higher than those of aqueous extracts. The IC$_{50}$ value of ethanolic extract were $264.20 \pm 17.90 \, \mu g/mL$ (DPPH) and $176.40 \pm 8.62 \, \mu g/mL$ (ABTS), respectively. In research of Lee et al. (2011), DPPH radical scavenging activity was increasing with increasing concentration of *P. sarmentosum*. IC$_{50}$ value of methanol extract was $129.65 \pm 1.04 \, \mu g/mL$ [25].

![Figure 2. The DPPH and ABTS radical scavenging activity antioxidant potential of ethanol and aqueous extracts of *P. sarmentosum* leaves.](image-url)
4. Conclusion

*P. sarmentosum* leaves may be regarded as strong sources of natural antioxidants as their extract are strongly antioxidant. Thus the plant could be known as food for antioxidants if eaten regularly and could prevent oxidative-related diseases. The findings of this analysis have shown that the extract of *P. sarmentosum* leaves contains biologically active ingredients such as alkaloids, coumarin, flavonoids, reducing compounds, saponins, tannins and terpenoids, which have a wide range of pharmacological properties. The ethanol extract from *P. sarmentosum* leaves had an average complete content of polyphenols (60.61 ± 0.96 µgGAE/mg) and a flavonoids content (70.14 ± 0.38 µgQE/mg). With that concentration of *P. sarmentosum*, DPPH and ABTS radical scavenging activity increased. The ethanolic extract provided the body with the necessary antioxidant nutrients required to strengthen the immune system, eliminate excess free radicals and stabilize the condition of oxidative stress.

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References

[1] Phan A N Q, Bach L G, Nguyen T D, Le N T H 2019 *J. Nanosci. Nanotechnol.* 19 974-978.
[2] N P Minh, Bach L G, M H Chau, L Y Loan, V T B Tram and T V Truyen 2019 *J. Pharm. Sci. & Res.* 11 279-283.
[3] Vo T T, T N Y Tran, T V L Nguyen, Tran A V and T T Tran 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* 736 062015.
[4] Nguyen T V L, M D Nguyen, D C Nguyen, Bach L G and Lam T D 2019 *Processes* 7 21.
[5] Sireeratatwong S, Vannasiri S, Sritiwong S, Itharat A and Jaijoy K 2010 *J. Med. Assoc. Thai.* 93 Suppl 7:S1-S6.
[6] Sundar U M, Ugusman A, Chua H K, Latip J and Aminuddin A 2019 *Front Pharmacol.* 2019 10:1033.
[7] Hematpoor A, Paydar M and Liew S Y 2018 *Chem Biol Interact.* 279 210-218.
[8] Hashim Fauzy F, Mohd Zainudin M, Ismawi H R and Elshami T F T 2019 *Evid Based Complement Altern Med.* 2019:7198592.
[9] Mohd Zainudin M, Zakaria Z and Megat Mohd Nordin N A 2015 *BMC Complement Altern Med.* 15 54.
[10] Subramaniam V, Adenan M I, Ahmad A R and Sahdan R 2003 *Malays J Nutr* 9 41–51.
[11] Azlina M F N, Qodriyah H M S, Akmal M N, Ibrahim I A A and Kamisah Y 2019 *Arch Med Sci.* 15 223-231.
[12] Nguyen N Q, Nguyen M T, Nguyen V T, Le V M, L H Trieu, Le X T, T V Khang, N T L Giang, N Q Thach and T T Hung 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* 736 022067.
[13] Tran T Y N, P T N Nguyen, Vo T T, D V Nguyen, V T Pham, Tran A V, Lam T D and T T Tran 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* 736 022064.
[14] Nguyen V T, Nguyen M T, N Q Nguyen, Mai H C, P M Quan, Bui L M, V M Le, V M Nguyen 2020 *Asian J. Chem.* 32(5) 1230-34.
[15] Ebrahimzadeh M A, Khalili M and Dehpour A A 2018 *Brac. J. Pharm. Sci.* 54.
[16] Nguyen V T, Nguyen M T, Tran Q T, P V Thinh, Bui L M, T H N Le, V M Le and H T K Linh 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* 736 022063.
[17] Nguyen V T, V M Le, T S Vo, Bui L M, H L T Anh and V T Danh 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* 736 062013.
[18] Nguyen M T, Nguyen V T, L V Minh, L H Trieu, Mai H C, Bui L M, Le X T and V T Danh 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* 736 062011.
[19] Nguyen M T, Nguyen V T, Le V M, L H Trieu, Lam T D, Bui L M, L T Nhan and V T Danh 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* 736 062012.
[20] Islam M, Jannat T, Kuddus Md R, Rashid M A and Haque M R 2019 L Clin. Phytosci. 5 42
[21] N P Minh, T H P Trang, N T T Trang, and Bach L G 2019 Res. Crops 20180-186.
[22] Nguyen N Q, L V Minh, L H Trieu, Bui L M, Lam T D, V Q Hieu, T V Khang and L N Y Trung 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 062017.
[23] P T N Trinh, T Q Nguyen, N V Hau, Q T Hung, C V Du, N T Tuan, N T L Thuy and L T Dung 2020 IOP Conf Series: Materials Science and Engineering 736 022080.
[24] Tran T Y N, P T N Nguyen, Vo T T, D C Nguyen, Lam T D, D V Nguyen, Tran A V, T T Tran and V T Pham 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 022065.
[25] Seyyedan A, Yahya F, Kamarolzaman M F F, Suhaili Z, Desa M N M, Khairi H M, Somchit M N, Fatimah C A, Teh L K, Salleh M Z and Zakaria Z A 2013 탑 3 19.1-19.32
[26] Lee K H, Padzil A M, Syahida A, Abdullah N and Zuhainis S W 2011 J. Med. Plants Res. 5 5555–63.