Studies on chemical, polyphenol content, flavonoid content, and antioxidant activity of sweet basil leaves (*Ocimum basilicum* L.)

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Abstract: Sweet basil (*Ocimum basilicum* L.) is commonly used as an attractive flavor in Vietnamese cuisine and a valuable remedy in traditional medicine. The purpose of this study is to analyze the phytochemical profile, as well as to calculate the content of polyphenols and flavonoids and antioxidant activity of ethanolic and aqueous extracts from sweet basil leaves. The findings revealed that the leaves of sweet basil contained a wide range of pharmacologically active substances, such as alkaloids, coumarins, tannins, flavonoids, sugars, phenols, terpenoids and saponins. The total phenolic and flavonoid contents of ethanolic extract achieved 29.60 ± 1.64 mg GAE/g and 19.58 ± 0.93 mg QE/g, respectively. Furthermore, ethanolic extract displayed good antioxidant activities as shown in DPPH and ABTS radical scavenging methods, represented by IC₅₀ of 91.31±4.28 µg/mL and 85.17 ± 3.91 µg/mL, respectively. These findings have shown that this plant is a potential source of natural antioxidants.

1. Introduction

Thanks to their nutritional value and minimal side effects on human health, medicinal plants have gained considerable public interest in recent years [1-7]. The genus *Ocimum* includes more than 150 species and is considered as one of the most abundant genera of *Lamiaceae* family. *Ocimum basilicum* L. (Sweet basil) is an annual herb distributing in a variety of regions around the world. The plant is commonly used in agricultural items and in oral treatment.

Thanks to the possible health effects, numerous plant antioxidant production plays an important role in various fields, preventing brain diseases, cancer, inflammation, disorder or neurodegenerative processes, diabetes, arthritis and cardiovascular disease [8-10]. Phenolic compounds are secondary metabolites of plants, such as flavonoid, alkaloid and terpenoid. These compounds play important biochemical roles and benefit for human well-being because of their antioxidant powers. Polyphenols can mitigate and reduce the harm of free radicals to the human body. The plant essential oil is also utilized as a perfumery. The leaves and flowering tops of sweet basil are used in herbal medicine as carminative, galactagogue and antispasmodic medicinal plants [11]. The scientific literature has extensively identified sweet basil as possessing biological properties such as antivirals, antifungal, antimicrobial, anti-allergic, anticancer, analgesic and immuno-stimulatory properties [12]. In the present study, sweet basil is one of the most common herbs consumed as a spice and is an abundance source of...
phenolic compounds especially phenolic acids, flavonol-glycosides and anthocyanins [13]. Extracts derived from spices have been documented to have antioxidant activity. [14]. However, there is no information on the free radicals scavenging activities of the aqueous and ethanol extracts from sweet basil.

Extraction is generally recognized as a mechanism of separating substances from raw plant materials [15]. Various techniques have been developed to obtain antioxidants from plants such as microwave and soxhlet extraction methods [16]. Nevertheless, the yield for extraction depends on the extraction process as well as several different conditions [17]. For this analysis, purified water and ethanol are the solvents chosen for the tests with different polarities [18]. Overall, due to current lack of knowledge, the present study aimed to analyze the phytochemical profile and determine the total contents of flavonoids and phenolics, as well as antioxidant activity of ethanol and aqueous extracts from O. basilicum L. leaves.

2. Materials and methods

2.1. Chemicals and reagents
Aluminium chloride, potassium persulfate, potassium acetate, sodium carbonate, methanol, ethanol, gallic acid, L-ascorbic acid, 2,2′-azinobis 3-ethylbenzothiazoline-6-sulphonate (ABTS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Folin-Ciocalteu reagent and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample preparation
Leaves from O. basilicum L. were collected from farms in Tay Ninh Province, Vietnam. The fresh leaves were dried under shade for 72 h at room temperature to eliminate the water content. The dried leaves were grounded into fine powder (1.0 mm) after drying, and stored until use at -20°C.

2.3. Solvent extraction
Dried sample of 10 g were extracted with 300 mL of ethanol 70% and distilled water at 60 °C for 1 h. After the extraction, the sweet basil leaves extract was then routed through a filter paper Whatman No. 1 and condensed at 45°C under reduced pressure (Heidolph, Germany). Aqueous and ethanolic extracts from the sweet basil leaves were subjected to determination of phytochemicals, TPC, TFC and antioxidant activity.

2.4. Phytochemical screening
Phytochemical analysis of ethanolic and aqueous extracts of sweet basil leaves were carried out using standard methods to detect secondary metabolites of the plant including of alkaloids, tannins, flavonoids, anthraquinons, saponins, triterpenoid and reducing sugars [19].

2.5. Total polyphenol content (TPC)
Determination of TPC followed a previously described procedure by Thuy et al [20]. Briefly, diluted sample was aspirated into a test tube, added with 10% Folin-Ciocalteu solution and homogenized with a Vortex machine. After 5 min, the mixture was introduced with 7.5% Na2CO3 solution, followed by 1-hour incubation in the dark. Absorbance measurement was at 765nm of wavelength using the UV-Vis spectrometer. The TPC of 1 g of dry extract was referenced to gallic acid as the standard (mg GAE/g).

2.6. Total flavonoid content (TFC)
Determination of TFC followed the previously described method by Mahboubi et al [17]. The extract was mixed with 10% AlCl3, 1M CH3COOK and distilled water and then vigorously shaken. Absorbance measurement was carried out at wavelength of 415 nm using the UV-Vis spectrometer. TFC of 1 g of dried extract was given by referring to quercetin as the standard (mg QE/g).
2.7. DPPH Scavenging Activity
The ability to scavenge DPPH of the extracts was evaluated using the method described by Thuy et al [20]. The working solution was prepared from stock solution diluted with methanol and had absorbance of 1.1 ± 0.02 at 517 nm. The working solution was then mixed with the extract, followed by 30 min of incubation in the dark. The absorbance of the sample and of the standard (methanol) were taken to determine DPPH scavenging activity as follows.

\[
\text{DPPH scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

Whereby \( A_A \) and \( A_C \) are the absorbance of the antioxidant and control, respectively.

2.8. ABTS Scavenging Activity
The ability to scavenge ABTS was evaluated using the method described by Thuy et al [20]. ABTS free radical solution was prepared by adding 7.4 mM ABTS solution to 2.6 mM \( \text{K}_2\text{S}_2\text{O}_8 \) solution and incubated in the dark for 24 h. The mixture was diluted with methanol to give absorbance value of 1.1 ± 0.02 at 734 nm. Working solution of ABTS was added to the diluted sample and incubated in the dark for 30 min. The control sample will be replaced by methanol. ABTS scavenging activity was determined based on the formula:

\[
\text{ABTS scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

Whereby \( A_A \) and \( A_C \) are the absorbance of the antioxidant and control, respectively.

2.9. Statistical Analyses
One-way ANOVA accompanied by Statgraphics Centurion XV version 15.0 and Fisher's Least Significant Difference (LSD) were used to analyze data. Differences for both measurements were found statistically relevant at \( P<0.05 \).

3. Results and discussion

3.1. Phytochemical analysis
Phytochemical analysis of ethanolic and aqueous extracts from sweet basil leaves were summarized in Table 1. By using the methods described in Ciulei I (1982), flavonoids, tannin and terpenoids have been detected in both extracts. Alkaloids were absent in aqueous extract while saponin and reducing sugar were not found in ethanolic extract (Table 1). Previous studies have shown that each of these compounds exhibited numerous important bioactivities. For instance, the polyphenol group is mainly responsible for defending against oxidative agents which affect some types of cell proteins and genetic materials [22]. Recent studies have confirmed the role of polyphenols in cancer prevention, cardiovascular diseases, diabetes mellitus, and neurodegenerative diseases linked to oxidative stress and chronic inflammation [23]. Saponins in the plant have the ability to protect liver function, anti-inflammatory, and against some viral diseases [24]. Terpenoids showed different activities like anticancer, antiviral, antibacterial and anti-inflammatory [25]. In modern medical studies, phenolic, alkaloid, flavonoid and terpenoid compounds are known as a source of potential substances for the study of natural compounds with biological activity used in prevention and treatment human diseases. Findings of phytochemical profiles of sweet basil leaf extracts could act as a preliminary work for further studies on measuring the exact amount of each compound, as well as optimizing the extraction process to achieve desirable yield.

| Table 1. Phytochemical screening of ethanolic and aqueous extracts of O. basilicum |  |  |
|---|---|---|
Chemical components | Ethanolic | Aqueous
---|---|---
Alkaloids | + | -
Tannins | + | +
Anthraquinones | - | -
Flavonoids | + | +
Terpenoids | + | +
Coumarins | + | -
Saponins | - | +
Reducing sugars | - | +

(+ ) indicates present, (−) indicates absence

Figure 1. Phytochemical analysis of *O. basilicum* leaves extracts. (a) Terpenoids test (b) Saponins test (c) Tannin test (d) Cyanidin test (e) Reducing sugars test (f) Alkaloids test.

3.2. TPC and TFC in different fractions

Phenolic compounds, often characterized by the phenolic rings and the structural elements which combine these rings to different classes, may include phenolic acids, flavonoids, stilbenes, tannins and lignans and are commonly detected in plants as secondary metabolites [26]. Plant-derived phenolics are known not only for their ability to resist oxidation but also as a stable intermediate metabolite. Flavonoids are the most prominent group of antioxidant compounds among plant phenolic compounds [27].

Table 2. TPC, TFC, and antioxidant activities (IC$_{50}$ values) of *O. basilicum* ethanolic and aqueous leaf extracts.

| Sample          | TPC (mg GAE/g) | TFC (mg QE/g) | IC$_{50}$ value (µg/mL) |
|-----------------|----------------|---------------|-------------------------|
|                 |                |               | DPPH                  | ABTS                      |
| Ethanolic extract | 29.60 ± 1.64$^b$ | 19.58 ± 0.93$^b$ | 91.31 ± 4.28$^b$ | 85.17 ± 3.91$^b$ |
| Aqueous extract  | 12.98 ± 0.53$^a$ | 5.77 ± 0.25$^a$ | 258.55 ± 6.08$^c$ | 223.13 ± 7.32$^c$ |
| Ascorbic acid   | −              | −             | 3.05 ± 0.28$^a$ | 2.51 ± 0.74$^a$ |

Results were derived from triplicate tests and were expressed as mean ± SD. Figures with different letters (a–d) in the same column are significantly different at p < 0.05.

TPC and TFC in the studied samples were determined and shown in Table 2. TPC of ethanolic extract is higher than aqueous extract with values of 29.60 ± 1.64 mg GAE/g and 12.98 ± 0.53 mg GAE/g, respectively. Similar observations where ethanolic extract contained more phenolics as compared with aqueous extract were also observed in *O. sanctum* (148.9 mg/g GAE and 90.4 ± 4.5 mg/g GAE) and *Tragopogon porrifolius* (145.3 ± 3.1 mg/g GAE and 102.9 ± 3.9 mg/g GAE) [28,29]. Likewise, TFC of
ethanolic basil leaf extract is also higher than aqueous extract with values of 19.58 ± 0.93 mg QE/g and 5.77 ± 0.25 mg QE/g, respectively. Considering those results, ethanol 70% is selected as the suitable solvent for more efficient phenolic extraction from sweet basil leaves.

3.3. Antioxidant activity

Many studies have shown that TPC and antioxidant activity are in strong correlation. Radical scavenging of DPPH and ABTS are two common methods for evaluating antioxidant activity in research [30]. The antioxidant capacity of the extracts is expressed by the IC50 value, which is the sample concentration inhibits 50% of free radicals. The lower the IC50 amount, the higher will be the antioxidant function.

Figure 2. Correlation between scavenged DPPH radicals and concentration of *O. basilicum* ethanolic (A) and aqueous extract (B).

Figure 2 showed the DPPH radical scavenging capability of ethanolic and aqueous extracts from *O. basilicum* leaves. In general, the antioxidant activity of ethanolic and aqueous extracts is closely related to the TPC of the sample. As shown by IC50 values of ethanolic (91.31 ± 4.28 μg/mL) and aqueous extracts (258.55 ± 6.08 μg/mL), the former showed a better scavenging effect on DPPH radicals than the latter. This is also compatible with previous research regarding the impact of extraction solvent on plant antioxidant activity [31].

Figure 3. Correlation between scavenged ABTS radicals and concentration of *O. basilicum* ethanolic (A) and aqueous extract (B).

The ABTS scavenging potential of ethanolic and aqueous extracts from the sweet basil leaves at varying concentrations was shown in Figure 3. Visually, ABTS scavenging activity was found to be proportional to the sample concentration. Furthermore, results from this assay were in close agreement with the DPPH assay performed earlier in the present study. Ethanol extract also showed higher antioxidant activity with IC50 value of 85.17 ± 3.91 μg/mL than aqueous extract with IC50 value of 223.13 ± 7.32 μg/mL. Similar result was also obtained in *T. porrifolius* extraction, in which the
antioxidant activity of 80% ethanolic extract were higher than that of aqueous extract [29]. Therefore, 70% ethanol/water solvent was chosen to extract the phenolic compounds from the sweet basil leaves.

4. Conclusion
The leaves of sweet basil contain most common plant compounds such as flavonoids, saponins, alkaloids, coumarins, tannins, reducing sugars, and terpenoids. Research results have also shown that ethanolic and aqueous extract of sweet basil leaves exhibited quite good antioxidant activity with relatively high total contents of polyphenols and flavonoids. Overall, results from the present study indicated that ethanol 70% is the most efficient solvent for basil leaves extraction and the plant could be considered as a potential resource of natural bioactive compounds for use in the food and nutraceutical industries.

5. References
[1] Mai H C, Le T T T, Diep T T, Le T H N, Nguyen D T and Bach L G 2018 Asian J. Chem. 30 293–7
[2] Nguyen P T N, Tran T H, Le T H N, Phan N Q A, Le T H, Nguyen T C T, Nguyen D T and Bach L G 2018 Solid State Phenom. 279 235–9.
[3] Phan A N Q, Bach L G, Nguyen T D and Le N T H 2019 J. Nanosci. Nanotechnol. 19 974–8
[4] Nguyen N Q, Nguyen M T, Nguyen V T, Le V M, Trieu L H, Le X T, Khang T V, Giang N T L, Thach N Q and Hung T T 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 022067
[5] Tran T Y N, Nguyen P T N, Vo T T, Nguyen D C, Lam T D, Nguyen D V, Tran A V, Tran T T and Pham V T 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 022065.
[6] Tran T Y N, Nguyen P T N, Vo T T, Pham V T, Tran A V, Lam D T and Tran T T 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 022064.
[7] Pham T N, X T Le, Nguyen P T N, Tran T H, Dao T P, Nguyen D H, Danh V T and Anh H L T 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 062005.
[8] Nguyen N Q, Le V M, Trieu L H, Bui L M, Lam T D, Hieu V Q, Khang T V and Trung L N Y 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 062017.
[9] Nguyen M T, Nguyen V T, Le V M, Trieu L H, Mai H C, Bui L M, Le X T and Danh V T 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 062011.
[10] Nguyen V T, Nguyen M T, Tran Q T, Thinh P V, Bui L M, Le T H N, Le V M and Linh H T K 2020 IOP Conf. Ser.: Mater. Sci. Eng. 736 022063
[11] Sekar K, Thangaraj S, Saravana B S, Harisaranraj R and Suresh K 2009 J. Phytol. 1 408–13.
[12] Gajula D, Varghese M, Boateng, Walker L T, Shackelford L, Mentreddy S R and Cedric S 2009 Int. J. Cancer 5 130–43.
[13] Phippen W B and Simon J E 1998 J. Agr. Food Chem. 46 1734–8.
[14] Gülçin I 2006 Toxicology 217 213–20.
[15] Zhang Q-W, Lin L-G and Ye W-C 2018 Chin. Med. 13
[16] Selvamuthukumaran M and Shi J 2017 Food Quality and Safety 1 61–81
[17] Belwal T, Pandey A, Bhatt I D and Rawal R S 2020 Sci Rep 10 917
[18] Nawaz H, Shad M A, Rehman N, Andaleeb H and Ullah N 2020 Braz. J. Pharm. Sci. 56 e17129
[19] Ciulei I (1982) Methodology for analysis of vegetable drug. Practical manual of industrial utilization of medicinal and aromatic plants (Bucharest: Ministry of chemical industry) p 67–85
[20] Thuy P H N, Tang N V, Quan V V, Bowyer M C and Scarlett C J 2017 J. Food Process. Pres. 41 e12879
[21] Mahboubi M, Kazempour N, and Nazar A R 2013 Jundishapur J. Nat. Pharm. Prod. 8 15–19.
[22] Marja P K, Anu I H, Heikki J V, Jussi-Pekka R, Kalevi P, Tytti S K and Marina H 1999 J. Agric. Food Chem. 47 3954–61.
[23] Ha L T N 2016 Vietnam J. Agri. Sci 14 1107–18
[24] Rahimi R, Ghiasi S, Azimi H, Fakhrani S and Abdollahi M 2009 Cytokine 49 123–9.
[25] Uddin G, Rauf A, Qaisar M, Rehman T U, Latif A and Ali M 2011 *Middle-East J. Sci. Res.* **8** 198–202.
[26] Manach C, Scalbert A, Morand C, Rémy C, Jiménez L 2004 *Am. J. Clin. Nutr.* **79** 727–47.
[27] Joon-Kwan M and Takayuki S 2009 *J. Agric. Food Chem.* **57** 1655–66.
[28] Mahajan N, Singh J and Sinha S 2014 *Int. J. Appl. Bio. Pharm. Tech* **5** 34–42
[29] Al-Rimawi F, Rishmuwi S, Ariqat S H, Khalid M F, Warad I and Salah Z 2016 *Evid. Based Complement. Altern. Med.* **2016** 1–7
[30] Jin D and Russell J M 2010 *Molecules* **15** 7313–52
[31] Nguyen V T, Nguyen M T, Nguyen N Q, Mai H C, Quan P M, Bui L M, Le V M and Nguyen V M 2020 *Asian J. Chem.* **32** 1230–34.

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