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HPLC profile of phenolic acids and flavonoids of *Ocimum sanctum* and *O. basilicum*

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ABSTRACT

This study aimed to investigate the antioxidant activity, total phenolic content (TPC), and total flavonoid content (TFC), as well as the phenolic profile of two species of *O. sanctum* (OS) and *O. basilicum* (OB). The TPC, TFC, and cultivated *sanctum*'s phenolic profiles were similar. The TPC of OS and OB produced 386 and 383 mg gallic acid equivalent (GAE) per 100 g, whereas 201 and 203 mg quercetin equivalent (QE) per 100 g of the extract was obtained during the TFC assay. The antioxidant activity of the extracts was determined by scavenging of DPPH radicals with an inconsiderable difference. HPLC techniques separated the individual phenolic acids and flavonoids. Phenolic acids (gallic, caffeic, ferulic, sinapic, and syringic) and flavonoids (quercetin, luteolin, rutin, apigenin, and kaempferol) were commonly identified and quantified in the chromatogram of OS and OB. The maximum gallic acid and quercetin content were found among phenolic acids and flavonoids. The maximum yield of quercetin was analyzed in both extracts.

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1. Introduction

Ocimum sanctum and *O. basilicum* belong to the Lamiaceae family; both are used as ornamental and culinary herbs. The genus *Ocimum* contains more than 150 species of herbs found in tropical regions of Asia and are rich sources of phytochemicals that contribute to many putative health benefits (Ali et al., 2014; Malekshahi et al., 2021; Mokat and Kharat, 2022; Shoker et al., 2021). Traditionally, it is used as a medicinal plant to treat cough, diarrhea, warts, worm, and kidney malfunctions. Externally, it is used as an ointment for insect bites and in the treatment of acne due to having antibacterial, anti-carcinogenic, antimutagenic, and antioxidant activity (Anantharam and Chittibabu, 2021; de Lima et al., 2014; Pandey et al., 2015; Sayyad et al., 2022; Shafqatullah et al., 2013). Numerous studies signify the strong antioxidant and biological activities of *Ocimum,* among other medicinal plants. The previous literature shows that Ocimum has a remarkable phenolic content yield (Gavrić et al., 2018; Kelm et al., 2000; Samson et al., 2007).

Phenolic compounds are the secondary plant metabolites that are widely distributed in plants. These compounds play an important role in health-promoting factors (Khatri et al., 2019; Scheublin et al., 2014). Scientific research suggests that these compounds have antioxidant properties that trap free radicals and reduce the risk of cancer, heart, and chronic diseases (Imram et al., 2014; Ssepuuya et al., 2021; Troise et al., 2014). These phenolic compounds have more than dozen of sub-classes but are mainly classified as simple and polyphenol (Matulja et al., 2022).

Phytonutrients like flavonoids have beneficial anti-inflammatory effects, and they protect your cells from oxidative damage that can lead to disease. These dietary antioxidants can prevent the

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development of cardiovascular disease, diabetes, cancer, and cognitive diseases like Alzheimer's and dementia.

Similarly, flavonoids, flavonoid polymers, and phenolic acids are most well-known due to having many advantageous features in the area where highly antioxidants and anti-inflammatory therapy are required to avoid diseases including cardiovascular diseases, diabetes, cancer, and cognitive diseases like Alzheimer's, and dementia (Khatri et al., 2019; Nemzer et al., 2021). These compounds are essential antioxidants due to their redox potential, which acts as a strong reducing agent, hydrogen donors, and single oxygen quenchers (Pisoschi et al., 2021). These compounds also possess metal chelating properties (Javanmardi et al., 2003; Qureshi et al., 2014; Tatipamula and Kukavica, 2021).

Due to many phenolic compounds in plants, quantifying and identifying each phenolic acid and flavonoid is tedious. Different techniques have been used for the separation and quantification of these compounds, wherein thin layer chromatography (TLC) and high-performance liquid chromatographic (HPLC) are the most widely used (Montedoro et al., 1992; Proestos et al., 2005; Qureshi et al., 2014).

The current study was designed to evaluate the TPC, TFC, and antioxidant activity of methanol extracts (obtained by cold extraction method) of *O. sanctum* and *O. basilicum* as well as the profile of phenolic acids and flavonoids using the HPLC technique.

Economic feasibility, extremely quick and efficient HPLC technique used in this increases the worth of the current study compared to some previously reported methods, such as TLC and HPTLC, etc.

2. Materials and methods

2.1. Collection of samples and materials

Greenish stems and leaves of the plant materials were collected separately from the botanical garden of PCSIR Labs complex Peshawar, brought to the Laboratory, washed thoroughly with tap water, and shade dried at room temperature. All solvents were of analytical grade and used as received.

2.2. Experimental procedure

The samples were extracted with methanol following a previously reported method with a slight modification (Khattak et al., 2007). Briefly, the collected leaves were air-dried at room temperature. Then, a 100 g powder sample was extracted with 98.8% methanol on an orbital shaker for 3 hours. The extract was then centrifuged at 6.000 rpm for 10 min. The supernatant was collected, and the organic solvent was removed by a sample concentrator in the presence of N_2 gas. The residues were then re-dissolved in 10 ml of methanol and stored for further analysis.

2.3. Determination of antioxidant activity

The antioxidant activity of OS and OB methanol extracts was determined by DPPH radical scavenging activity (Ashif and Ullah, 2013). 1 ml of each extract was diluted with 9 ml methanol and centrifuged for 5 min at 6.000 rpm. 1ml of the supernatant was added with 2 ml of DPPH methanol solution (0.004%, w/v). The absorbance was measured at 517 nm against the blank after 30 minutes of incubation. The scavenging activity of the extract was determined in percent using the following equation.

$$\%\,Scavenging\ Activity = \frac{Abs\ of\ control\ -\ Abs\ of\ sample}{Abs\ of\ the\ control} \times 100$$

2.4. Determination of total phenolic content (TPC)

The TPC of methanol extract OS and OB was carried out using Folin-Ciocalteau Reagent (FCR) (Meda et al., 2005). 100 µg extract was diluted with 400 µl of deionized water and 2ml of 7.5 (m/v) sodium carbonate solution. Then, the resultant solutions were mixed with 2.5 ml of 0.2M Folin-Ciocalteu reagent and incubated for 2h. The absorbance was measured against blank at 760 nm using a UV/Vis spectrophotometer, and the TPC was measured from the standard curve of six different concentrations of gallic acid. The result was expressed as mg of gallic acid equivalents per gram dry weight of leaves.

2.5. Determination of total flavonoid content (TFC)

The TFC was analyzed by the spectrophotometric method (Boateng et al., 2008). 1 ml of each methanol extract was diluted with 4 ml of deionized water and 300 µl of sodium nitrate solution (15 g / 100 ml), 4 ml of sodium hydroxide solution (4 mg / 100 ml), and 300 µl of methanol aluminum chloride (10 g / 100 ml) was added and made the final solution up to 10 ml with distilled water. The absorbance was then measured at 510 nm after 15 minutes. This method is based on the reaction between aluminum chloride and flavonoids. The TFC was measured from the standard curve of six different concentrations of quercetin and expressed as mg of quercetin equivalent per gram of dry weight.

$$Y = 0.052x + 0.049, R_2 = 0.999$$

2.6. Determination of phenolic acids and flavonoids by HPLC

Determination of phenolic acids and flavonoids was carried out by HPLC (Hitachi D-2000) using a UV-Vis detector (L-2420). The phenolic acids (gallic, syringic, vanillic, and caffeic acid) were detected at 280 nm while flavonoids (quercetin, luteolin, rutin, apigenin, and kaempferol) at 360 nm according to the reported method (Pająk et al., 2014). The chromatographic separation was achieved using Intersil column ODS-3 C18 (5 µm, 250 × 4.6 mm GL Science Inc. Tokyo, Japan) at 30 °C. The chromatographic analysis was conducted with gradient elution using two solvents. Solvent-A: acidified water (2.5 g Acetic acid / 100ml) and Solvent-B: methanol. A linear gradient was applied for the first 10 minutes, with an increase of mobile phase B from 3 to 8%. After 10 minutes, the mobile phase B increased to 15, 20, 30, and 40% at 20, 30, 40, and 50 minutes, respectively. The quantification of phenolic acid and flavonoids was carried out using the external standard method.

3. Results and discussion

3.1. Antioxidant activity, total phenolic, and total flavonoid contents

The antioxidant activity, TPC, and TFC of OS and OB are given in Table 1. The content was evaluated by scavenging of free radical of DPPH. The antioxidant molecules in the extract quench these free radicals and convert them to colorless 2,2 diphenyl-1-hydrazine resulting in the decrease in absorbance of the DPPH solution. The OS and OB scavenged 61 and 60% of the DPPH free radicals.

The TPC and TFC of *O. sanctum* and *O. basilicum* are determined as GAE and QE, presented in Table 1. The TPC content was found as 386 mg GAE/100 g in *O. sanctum* and 383 mg GAE/100 g in *O. basilicum*, while the TFC was 201.6 and 203.4 mg QE/100 g. Plants

are a good source of phenolic compounds, and the study by Javanmardi et al. (2003) showed that the TPC of 20 medicinal plants was in the range of 2.34-152.32 mg GAE/100g. However, we found TPC too high in both *Ocimum* species compared to the abovementioned authors. These results agree with Naithani et al. (2006) and Kähkönen et al. (1999) that the TPC and TFC depend upon the extraction procedure and solvent. The authors found that these two *Ocimum* species have a very close relation to the phytochemicals.

Table 1. Antioxidant activity, total phenolic and flavonoid contents of *O. sanctum* and *O. basilicum*

Sample	% Scavenging (DPPH)	Content (mg / 100 g)	
		Total phenolic	Total flavonoid
O. sanctum	61.4 ± 1.2*	386 ± 2.5	201.6 ± 1.32
O. basilicum	60.56 ± 1.5	383 ± 2.4	203.4 ± 1.43

*RSD: relative standard deviation; the number of replicates: *n* = 3

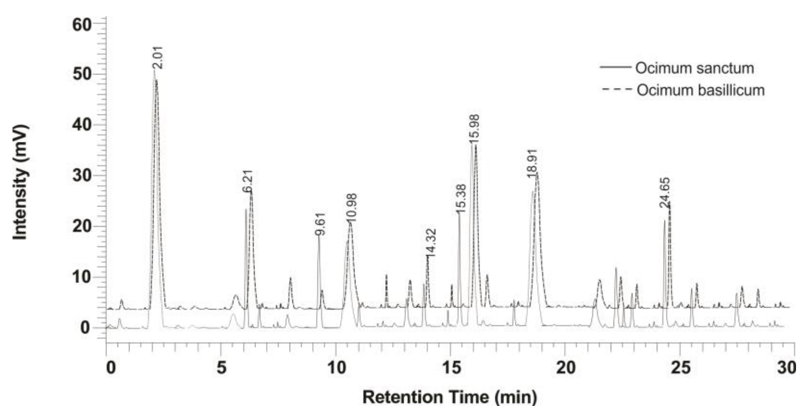

Figure 1. HPLC profile of OS and OB [Detection: 230 nm, Peaks at retention time: 2.01 (gallic); other unidentified compounds]

Table 2. Phenolic acid profile of *O. sanctum* and *O. basilicum*

Sample	Phenolic acid (mg / 100 g)					Total
	Gallic	Caffeic	Ferulic	Sinapic	Syringic	
O. sanctum	5.5 ± 0.8*	8.1 ± 0.6	5.3 ± 1.2	3.4 ± 0.8	2.1 ± 0.5	56.4
O. basilicum	6.5 ± 1.0	7.9 ± 0.5	4.9 ± 1.9	4.6 ± 0.7	1.8 ± 0.3	58.1

*RSD: relative standard deviation; the number of replicates: *n* = 3

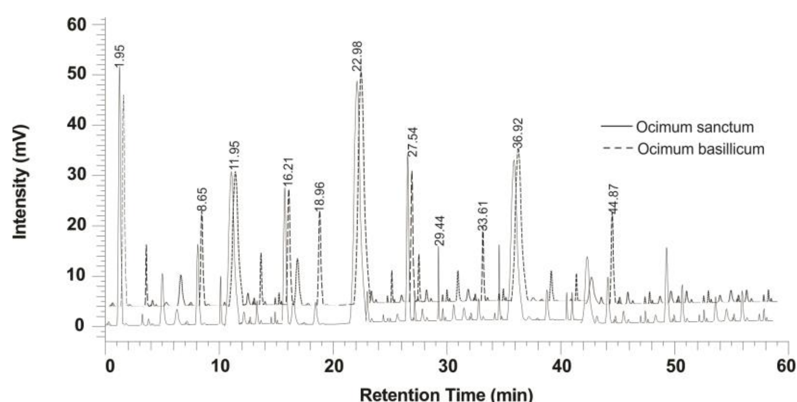

Figure 2. HPLC profile of OS and OB [Detection: 230 nm, Peaks at retention time: 2.01 (gallic); other unidentified compounds]

Table 3. Flavonoid profile of *O. sanctum* and *O. basilicum*

Sample	Flavonoid (mg / 100 g)					Total
	Quercetin	Luteolin	Rutin	Apigenin	Kaempferol	
O. sanctum	2.1 ± 0.3*	1.6 ± 0.7	1.6 ± 0.2	1.8 ± 0.1	1.9 ± 0.1	19.0
O. basilicum	3.4 ± 0.3	1.5 ± 0.5	1.9 ± 0.1	2.3 ± 0.2	1.1 ± 0.2	19.2

*RSD: relative standard deviation; the number of replicates: *n* = 3

3.2. The profile of phenolic acids and flavonoids

In this study, free phenolic acids were extracted and determined in *O. sanctum* and *O. basilicum*. Some other phenolic acids, such as esters, glycosides, and bound complexes, are also present in plants. Five phenolic acids (gallic, caffeic, sinapic, ferulic, and syringic) were identified in the HPLC chromatogram shown in Figure 1, and the quantitative analysis is presented in Table 2. The caffeic and gallic acid content was found in maximum yield in both the samples (8.1 and 5.5 mg / 100 g in *O. sanctum*, while 6.5 and 7.9 mg / 100 g in *O.*

basilicum). The ferulic, chlorogenic, and syringic content was 5.3, 3.4, and 2.1 mg / 100 g in *O. sanctum* and 4.9, 4.6, and 1.8 mg / 100 g in *O. basilicum*, respectively.

In our previous study, we had identified and quantified the five phenolic acids, including gallic and syringic, in methanol extract of *O. sanctum* by different extraction procedures and methods, in which syringic acid was not detected while gallic acid content was 2.97 and chlorogenic was 1.86 mg / 100 g, which is in close agreement with a previous study (Jayasinghe et al., 2003). Using the aforementioned method, sinapic acid was not detected in any sample. On the other hand, five flavonoids were identified in the HPLC chromatogram shown in Figure 2, and the content of flavonoids was quantified in Table 3.

The quercetin content was found to be maximum in both samples, 2.1 mg / 100 g in *O. sanctum* and 3.4 mg / 100 g in *O. basilicum*, while the rest of luteolin, rutin, apigenin, and kaempferol were 1.6, 1.6, 1.8, and 1.9 mg /100 g in *O. sanctum* and 1.5, 1.9, 2.3, and 1.1 mg / 100 g in *O. basilicum* (Lee and Scagel, 2009).

4. Conclusions

This protocol represents a comparative study of natural antioxidants, for example, phenolic acid and flavonoid content determination of two potential medicinal plants. Both OS and OB were potential sources of natural antioxidants. The plants were rich in active components such as phenolic acids, gallic, caffeic, ferulic, sinapic, syringic, and flavonoids, including quercetin, luteoline, and rutin apigenin, and kaempferol. The TPC content was 386 mg GAE / 100 g in *O. sanctum* and 383 mg GAE / 100 g in *O. basilicum*, whereas the TFC was 201.6 and 203.4 mg QE / 100 g on average. Further, OS and OB scavenged 61% and 60% of the DPPH free radicals. As previously described, syringic acid was not detected in our previous investigations, although the used protocols were considerably identical. Therefore, further analysis is suggested to verify and purify the biologically active components of the plants above to optimize the production of therapeutic contents for their ultimate implementation in the food industry, such as herbal antioxidants, etc.

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None.

Conflict of interest

The authors confirm that there are no known conflicts of interest.

CRediT authorship contribution statement

Shafqat Ullah: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing, Review & Editing
Naseem Rauf: Data curation, Funding acquisition, Project administration, Software, Validation, Writing
Arshad Hussain: Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Writing, Review & Editing
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Supplementary File

None.

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