Total Phenolic and Flavonoid Contents, and Antioxidant Activity from the Root Extract of Cannabis sativa L

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Abstract

This study investigates the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *Cannabis sativa* roots (Siskiyou Gold strain) extracted sequentially using solvents with increasing polarity: hexane, ethyl acetate, and methanol. The methanol extract (SG-MET) provided the highest yield (9.53%) compared to other solvents. The results demonstrate that solvent polarity significantly influences the extraction efficiency of phenolic and flavonoid compounds. Methanol, a highly polar solvent, extracted the highest total phenolic content (36.18 ± 1.01 mg GAE/g extract) and comparable total flavonoid content (36.79 ± 1.38 mg QE/g extract). Additionally, the methanol extract exhibited the strongest antioxidant activity (IC_{50} = 554.82 ± 5.52 µg/mL) among the three extracts, although it remained lower than the standard Trolox (IC_{50} = 5.96 ± 0.13 µg/mL). These findings highlight methanol's effectiveness in extracting polar bioactive compounds, particularly phenolics and flavonoids, from *Cannabis sativa* roots. The study suggests that the methanol extract has significant potential as a source of bioactive compounds with antioxidant properties, which could be further explored for applications in pharmaceuticals, nutraceuticals, and related fields.

Keywords: Cannabis, Roots, phenolic contents, flavonoid contents, Antioxidant

1. Introduction

Cannabis is an annual herbaceous plant belonging to the family Cannabidaceae, with the scientific name *Cannabis sativa L. subsp. Indica*. It is commonly referred to as cannabis, marijuana, ganja, or sometimes as Indian hemp. Cannabis is believed to have originated in Central Asia and has since spread and been cultivated in many parts of the world. It is an economically and medicinally significant plant due to its diverse bioactive compounds, such as cannabinoids, terpenoids, phenolics, and flavonoids, which play important roles in its biological properties (Andre et al., 2016; Brenneisen, 2007).

Siskiyou Gold is a cannabis strain renowned for its distinctive characteristics, making it popular among both recreational and medical users. Known for its ability to induce relaxation, reduce stress, and alleviate pain, this strain is the result of a carefully curated crossbreeding process designed to achieve a balanced cannabinoid and terpene profile. Siskiyou Gold stands

out with its sweet, fruity aroma combined with floral undertones, offering a rich and enjoyable sensory experience.

Often associated with relaxation and tranquility, Siskiyou Gold is commonly used to promote restful sleep, reduce inflammation, and provide relief for chronic pain and stress. Its unique blend of effects and flavors makes it a preferred choice for those seeking a well-rounded cannabis experience (Siskiyou Seeds, 2021; Cannabis Reports, 2023). In addition to cannabinoids, phenolic and flavonoid compounds are particularly well-known as potent antioxidants that help reduce the risk of chronic diseases, such as cancer, cardiovascular diseases, and inflammation (Pietta, 2000). However, studies on these compounds in different parts of the cannabis plant, particularly in the roots, remain limited. A comprehensive exploration of the roots may lead to novel discoveries and further applications.

The roots of cannabis have been of interest in traditional medicine, where they have been used to treat various ailments, such as inflammation, infections, and pain (Bouquet, 1950). Although there have been some studies on the chemical composition of cannabis roots, data on the total phenolic and flavonoid contents in the roots of *Cannabis sativa* L. remain scarce. Evaluating the phenolic and flavonoid contents in the roots of this plant is essential for identifying its chemical properties and potential bioactive benefits. Therefore, this study aims to analyze the total phenolic and flavonoid contents in the roots of *Cannabis sativa L.* to provide fundamental data for the development and utilization of these compounds in medicine, the nutraceutical industry, and future research.

2. Research Objective

1. To investigate the total phenolic and total flavonoid contents in the root extract of Cannabis sativa L.

2. To evaluate the antioxidant activity of the root extract of Cannabis sativa L.

3. Materials and Methods

3.1 Preparation of Extracts

The roots of *Cannabis sativa* (Siskiyou Gold strain) were collected from Tenrain Co., Ltd., No. 15 Moo 6, Charoentham, Wihan Daeng District, Saraburi 18150, Thailand, in December 2022. The roots were air-dried, yielding a dry weight of 150 grams, and ground into a fine powder. The powdered root material was extracted using hexane as the solvent. The extract was filtered through Whatman Qualitative Filter Paper No. 1, and the solvent was evaporated using a rotary evaporator, yielding the hexane fraction. The residue remaining after hexane extraction (10.4 grams) was soaked in a more polar solvent, ethyl acetate. The extract was filtered, and the solvent was evaporated to obtain the ethyl acetate fraction (9.9 grams). The remaining residue from the ethyl acetate extraction was subsequently soaked in an even more polar solvent, methanol. The extract was filtered, and the solvent was evaporated, yielding the methanol fraction (14.3 grams). This sequential extraction process yielded three fractions: hexane, ethyl acetate, and methanol.

3.2 Determination of the total phenolic content

To determine the total phenolic content, 20 µL of the *Cannabis sativa* root extract (Siskiyou Gold Strain) was mixed with 100 µL of 10% Folin–Ciocalteu reagent in a 96-well plate. Then, 80 µL of 7% sodium carbonate was added to the mixture. The mixture was incubated for 30 min at room temperature in the dark. After incubation, absorbance was measured at 760 nm. The total phenolic content is expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). The procedure was performed in triplicate (Alzagameem, A., et al., 2020; Folin, O., & Ciocalteu, V., 1927).

3.3 Determination of the total flavonoid content

To determine the total flavonoid content, a 2% aluminum chloride ($AlCl_3$) solution was mixed with the extract sample at a 1:1 ratio in a 96-well plate. The mixture was incubated for 20 min at room temperature. After incubation, absorbance was measured at 415 nm. The results are expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g extract). The analysis was performed in triplicate (Chang, C., et al., 2002, Panyatip, et al., 2022). The total phenolic and flavonoid contents of *Cannabis sativa* root extract (Siskiyou Gold Strain) are reported as mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test, and the t-test ($p < 0.05$), using SPSS Statistics version 26.

3.4 Evaluation of Antioxidant Activity Using the DPPH Assay

The DPPH assay was conducted as different concentrations of extracts were mixed with equal proportions of DPPH solution in a 96-well plate. The plate was incubated in the dark for 30 min. After incubation, absorbance was measured at 517 nm, with Trolox serving as the positive control. The percentage of antioxidant activity (% inhibition) and the IC_{50} were calculated from these measurements. Each assay was conducted in triplicate to ensure reproducibility and accuracy (Sripan, P., et al., 2022).

4. Results and discussion

This study aimed to investigate the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH radical scavenging activity) of *Cannabis sativa* root extracts (Siskiyou Gold strain) using solvents with varying polarity hexane, ethyl acetate, and methanol. Dried roots were sequentially extracted, yielding three fractions (**Table 1**), hexane extract (SG-HEX), ethyl acetate extract (SG-EtOAC), and methanol extract (SG-MET). The extraction yields were 10.4 g for SG-HEX, 9.9 g for SG-EtOAC, and 14.3 g for SG-MET. When calculated as percentages of the dry root weight, the extraction yields were 6.93%, 6.60%, and 9.53%, respectively. These findings demonstrate a significant difference in extraction efficiency depending on the solvent polarity. Methanol, a highly polar solvent, yielded the highest extraction efficiency (9.53%), highlighting its ability to dissolve polar compounds such as phenolics and flavonoids, which are commonly found in plant roots. In contrast, hexane, a non-polar solvent, produced the lowest yield (6.93%), reflecting its limited capacity to extract polar bioactive compounds. The results underscore the critical role of solvent polarity in extracting bioactive compounds from *Cannabis sativa* roots, suggesting that methanol is particularly effective for phenolic and flavonoid extractions. These findings provide valuable insights for selecting solvents in future studies and for the development of bioactive compound extraction protocols.

Table 1 Extraction Yield from Cannabis sativa Roots (Siskiyou Gold Strain)

Samples	Yield (g)	Yield (%)
SG-HEX	10.4	6.93
SG-EtOAC	9.9	6.60
SG-MET	14.3	9.53

Note: SG-HEX=Hexane extract, SG-EtOAC=Ethyl acetate extract and SG-MET: Methanol extract

The total phenolic content (TPC) of *Cannabis sativa* root extracts (Siskiyou Gold strain) was analyzed and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). The results are shown (**Table 2**) the SG-HEX extract exhibited the lowest TPC at 6.24 ± 0.47 mg GAE/g extract, the SG-EtOAC extract showed a moderate TPC of 11.91 ± 0.65 mg GAE/g extract and the SG-MET extract demonstrated the highest TPC at 36.18 ± 1.01 mg GAE/g extract. These findings indicate that phenolic compounds are more effectively extracted by polar solvents such as methanol, which aligns with the polarity of phenolic compounds. The significantly higher TPC in the methanol extract (SG-MET) suggests that polar phenolic compounds, commonly found in plant roots, are efficiently dissolved and extracted by highly polar solvents. This pattern emphasizes the critical role of solvent polarity in the extraction process. The low TPC observed in the hexane extract (SG-HEX) reflects the limited solubility of phenolic compounds in non-polar solvents, such as hexane. In contrast, the intermediate TPC in the ethyl acetate extract (SG-EtOAC) is consistent with its medium polarity, which can extract some polar compounds but not as effectively as methanol.

The total flavonoid content (TFC) was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). The results are as follows (**Table 2**) SG-HEX: 32.73 ± 0.34 mg QE/g extract, SG-EtOAC: 36.55 ± 0.09 mg QE/g extract and SG-MET: 36.79 ± 1.38 mg QE/g extract. The TFC values for SG-EtOAC and SG-MET were slightly higher than that of SG-HEX, indicating that flavonoids, which generally have moderate polarity, dissolve effectively in both moderately polar solvents (ethyl acetate) and highly polar solvents (methanol). The small difference in TFC between SG-EtOAC and SG-MET suggests that flavonoids are well-extracted in a range of polarities.

Table 2 Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity of Cannabis sativa Root Extract (Siskiyou Gold Strain)

Samples	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	DPPH; IC_{50} (µg/ml)
SG-HEX	6.24 ± 0.47	32.73 ± 0.34	>1000
SG-EtOAC	11.91 ± 0.65	36.55 ± 0.09	609.31 ± 43.09
SG-MET	36.18 ± 1.01	36.79 ± 1.38	554.82 ± 5.52
Trolox			5.96 ± 0.13

The antioxidant activity of the *Cannabis sativa* root extracts (Siskiyou Gold strain) was evaluated using the DPPH radical scavenging assay. The results are expressed as IC_{50} values (µg/mL), which represent the concentration of extract required to inhibit 50% of DPPH

radicals. The results are SG-HEX was IC_{50} > 1000 µg/mL, indicating very low antioxidant activity, SG-EtOAC and SG-MET (IC_{50} = 609.31 ± 43.09 and 5.96 ± 0.13 µg/mL. The results demonstrate significant differences in antioxidant activity among the three extracts. The methanol extract (SG-MET) exhibited the best antioxidant activity (IC_{50} = 554.82 µg/mL), followed by the ethyl acetate extract (SG-EtOAC), while the hexane extract (SG-HEX) showed negligible activity with IC50 > 1000 µg/mL. Compared to Trolox, a standard antioxidant with much lower IC_{50} (5.96 ± 0.13 µg/mL), all three extracts showed considerably weaker antioxidant activity. The higher antioxidant activity observed in the methanol extract (SG-MET) is likely due to its higher total phenolic and flavonoid contents, as phenolic compounds are known to act as effective antioxidants by donating hydrogen atoms to neutralize free radicals (Chang et al., 2002; Pietta, 2000). These findings indicate that solvent polarity influences not only the extraction efficiency but also the antioxidant potential of the extracts. Methanol, being a highly polar solvent, efficiently extracts phenolic and flavonoid compounds with strong radical-scavenging activity. In contrast, hexane, a non-polar solvent, was ineffective in extracting such bioactive compounds, resulting in weak antioxidant activity.

The results demonstrate that solvent polarity significantly affects the extraction efficiency of phenolic and flavonoid compounds. Methanol (SG-MET), a highly polar solvent, extracted the highest phenolic content (36.18 ± 1.01 mg GAE/g extract) and comparable flavonoid content (36.79 ± 1.38 mg QE/g extract). The findings suggest that methanol is the most effective solvent for extracting polar bioactive compounds from *Cannabis sativa* roots. The methanol extract (SG-MET) displayed the highest antioxidant activity among the three extracts but still fell significantly short of the Trolox standard. These results suggest that *Cannabis sativa* root extracts contain bioactive compounds with moderate antioxidant properties, particularly in highly polar solvents like methanol.

5. Conclusion

This study investigates the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of Cannabis sativa roots (Siskiyou Gold strain) extracted sequentially using solvents of increasing polarity: hexane, ethyl acetate, and methanol. The methanol extract (SG-MET) provided the highest yield (9.53%) compared to other solvents. The methanol extract (SG-MET) exhibited the highest total phenolic content (36.18 ± 1.01 mg GAE/g extract). The ethyl acetate extract (SG-EtOAC) and methanol extract (SG-MET) showed similar flavonoid content. Antioxidant Activity: The methanol extract (SG-MET) demonstrated the strongest antioxidant activity (IC_{50} = 554.82 ± 5.52 µg/mL), although it remained significantly lower than the Trolox standard (IC_{50} = 5.96 ± 0.13 µg/mL). This study highlights that the methanol extract has the greatest potential as a source of phenolic and flavonoid compounds, exhibiting the best antioxidant activity among the tested extracts. These findings provide a solid foundation for further research into developing bioactive compounds for potential applications in pharmaceuticals and nutraceuticals.

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