The Pre-Harvest Flushing Effect on The Phytochemical Composition and Antioxidant Activity of Cannabis sativa Fluorescence, Leaves and Twigs

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Abstract

Cannabis (*Cannabis sativa* L.) contains many important bioactive compounds such as terpenes and cannabinoids (THC and CBD). These compounds were used in medical benefits and recreational uses. During the cultivation process, proper care throughout the growth cycle is crucial. One key step is nutrient flushing before harvest which involves rinsing nutrients from the plant's growing medium by watering with plain water. This process was typically done 2-3 weeks before harvesting. Therefore, this research aims to investigate the chemical composition using GCMS techniques. Also, total phenolic, flavonoid, tannin, chlorophyll and carotenoid contents were determined. The antioxidant activity of the fluorescence, leaves, and twigs from flushed and unflushed before harvest were analyzed. The results showed that the flushing process affected to chemical composition which are cannabinoids and triterpenes in twigs and didn't affect to fluorescence and twigs. Moreover, this process increased total phenolics, flavonoids and tannin in fluorescence. Also increased total flavonoids, tannin and chlorophyll in twigs and increased total carotenoid in leaves. For antioxidant activity, the IC_{50} of fluorescence, leaves and twigs were increased by DPPH assay and FRAP value of fluorescence and twig were increased. Therefore, it can be concluded that the flushing before harvest affects the chemical composition and antioxidant activity, which may be applied to the cultivation of other plants.

Keywords: Antioxidant activity, Cannabis, Chemical composition, Flushing

1.Introduction

Cannabis sativa L. has emerged as a subject of substantial scientific interest due to its diverse therapeutic applications and pharmacological properties [1]. Pre-harvest flushing, a cultivation technique involving the systematic reduction or complete elimination of nutrient supply prior to harvest, has been widely implemented by cannabis cultivators to purportedly enhance product quality [2]. However, empirical evidence regarding its impact on the phytochemical profile and bioactive properties remains limited.

The chemical constituents of cannabis encompass a complex array of bioactive compounds, including cannabinoids, terpenes, flavonoids, and phenolic compounds, which collectively contribute to its therapeutic efficacy and antioxidant properties [3]. These compounds exhibit differential distribution patterns across various plant tissues, including flowers, leaves, and twigs, with distinct concentration gradients and compositional variations [4]. The antioxidant capacity of cannabis has been primarily attributed to its phenolic compounds and flavonoids, which demonstrate significant free radical scavenging activity and oxidative stress mitigation potential [5].

Although numerous studies have examined the phytochemical profiles of cannabis under varying cultivation parameters [6], the specific effects of pre-harvest flushing on these compounds remain inadequately characterized. Elucidating the relationship between flushing protocols and the plant's chemical composition and antioxidant properties is essential for optimizing cultivation methodologies and maximizing the therapeutic potential of cannabis-derived products.

1.2Research Objective

The research aims to investigate the chemical composition using GCMS techniques. Also, total phenolic, tannin, flavonoid, chlorophyll and carotenoid contents were determined. The antioxidant activity of the fluorescence, leaves, and twigs from flushing and non-flushing before harvest were analyzed by DPPH, ABTS and FRAP assays.

2.Material and methods

2.1Cannabis cultivation and sample preparation

Cannabis sativa was cultivated in indoor cultivation room. Before harvest, the *C. sativa* tree was separated into two group which are flushing and non-flushing. The *C. sativa* fluorescence, leaves and twigs of each group were cut and mashed to powder. Each part was extracted with ethanol by sonication technique for 15 min (3 time). The extract was filtrate and evaporated by using rotary evaporator to obtain fluorescence flushing (FF), fluorescence non-flushing (FN), leaves flushing (LF), leaves non-flushing (LN), twigs flushing (TF) and twigs non-flushing (TN).

2.2Phytochemical analysis

The phytochemical analysis was investigated the chemical composition in cannabis extract by GCMS which used the GCMS condition follower by Palmieri, et al., 2021 [7]. Also, total phenolic and tannin contents were used the Folin-Ciocalteu assay, total flavonoid content was used the aluminum chloride colorimetric assay were described by Sripan et al., 2022 [8] and Champatasi et al., 2022 [9]. Total chlorophyll and carotenoid contents assays were described by Arnon, 1949 [10] and Momin and Kadam, 2011 [11].

2.2Antioxidant activity

The antioxidant activity was used DPPH, ABTS and FRAP assay. Trolox was used as a positive control. These methodologies were described by Yongram et al., 2025 [12].

2.3Statistical analysis

The results were presented as mean ± SD. The statistical analysis was done by one-way ANOVA with Tukey HSD and t-Test to compare the differences between sample groups. The differences were considered to be significant at $p<0.05$ using SPSS 23.0 software for Windows. (SPSS Inc., Chicago, IL, USA).

3.Results

3.1Phytochemical analysis

The chemical composition of *C. sativa* fluorescence, leaves, and twigs from flushing and non-flushing was used the GCMS. The result showed that the 7 chemical group which are cannabinoids, monoterpenes, diterpenes, sesquiterpenes, phytosterols, triterpenes and miscellaneous in Table 1. The Flushing process had an effect on the cannabinoids group in fluorescence and phytosterols, triterpenes and miscellaneous group in twigs.

Table 1: The chemical composition of C. sativa fluorescence, leaves, and twigs from flushing and non-flushing

Chemical group	% Peak area					
	FF	FN	LF	LN	TF	TN
Cannabinoids	98.84	98.51	94.94	93.91	55.28	69.13
Monoterpenes	0.26	0.28	0.11	0.06	0.00	0.00
Diterpenes	0.00	0.00	0.69	0.56	0.20	0.00
Sesquiterpenes	0.74	0.95	1.05	1.36	0.08	0.00
Phytosterols	0.00	0.00	0.61	0.51	16.81	9.90
Triterpenes	0.00	0.09	1.21	2.03	10.85	6.97
Miscellaneous	0.00	0.00	0.98	0.84	11.43	5.05

Note: FF as fluorescence flushing, FN as fluorescence non-flushing, LF as leaves flushing, LN as leaves non-flushing, TF as twigs flushing and TN as twigs non-flushing.

The total phenolic, flavonoid, tannin, chlorophyll and carotenoid contents showed in Table 2 and 3. In Fluorescence, the flushing process affects total phenolic, flavonoid and tannin contents. For leaves, its process influences total chlorophyll and carotenoid contents and also affects total flavonoid, tannin and chlorophyll contents in twigs.

3.2Antioxidant activity

The results showed the *C. sativa* extract demonstrated a good antioxidant activity in several part via DPPH, ABTS and FRAP assays. The Fluorescence had a higher antioxidant activity than the other part which is FF showed the highest antioxidant activity in ABTS. However, Flushing process effect to antioxidant in fluorescence by ABTS assay, including fluorescence and twigs by FRAP assay in Table 4.

Table 4: The Antioxidant activity of C. sativa

Part of plant	DPPH; IC_{50} (µg/ml)		ABTS; IC_{50} (µg/ml)		FRAP (mmol Fe^{2+}/100g extract)	
	Flushing	Non-flushing	Flushing	Non-flushing	Flushing	Non-flushing
Fluorescence	57.54 ± 2.01^{bA}	43.99 ± 0.43^{bA}	7.84 ± 0.36^{aA}	8.13 ± 0.63^{aA}	53.53 ± 2.66^{bA}	44.25 ± 2.67^{bB}
Leaves	144.36 ± 2.45^{dA}	122.88 ± 5.51^{cA}	19.24 ± 1.20^{bA}	16.70 ± 1.01^{bA}	45.20 ± 4.54^{bB}	56.76 ± 2.82^{bA}
Twigs	308.38 ± 1.20^{fA}	286.82 ± 10.24^{eA}	37.43 ± 0.26^{dB}	31.14 ± 1.79^{cA}	38.16 ± 1.15^{bA}	31.97 ± 0.19^{bB}
Trolox	6.24 ± 0.05^{a}		5.31 ± 0.08^{a}		1932.27 ± 42.38^{a}	

Note: Capital letters indicate a significant difference between flushing and non-flushing and small letters indicate a significant difference part of plant at $p<0.05$.

Table 2: The total phenolic and flavonoid contents of C. sativa

Part of plant	TPC (mg GAE/g extract)		TFC (mg QE/g extract)		Tannin content (mg TAE/g extract)	
	Flushing	Non-flushing	Flushing	Non-flushing	Flushing	Non-flushing
Fluorescence	79.62 ± 4.68^{aA}	73.06 ± 5.42^{aA}	44.54 ± 0.46^{aA}	43.46 ± 0.28^{aA}	109.60 ± 5.96^{aA}	61.40 ± 0.48^{aB}
Leaves	47.43 ± 2.56^{bA}	50.56 ± 1.01^{bA}	36.94 ± 0.73^{bA}	43.03 ± 0.39^{aA}	47.29 ± 1.06^{bB}	59.70 ± 1.14^{aA}
Twigs	41.62 ± 2.15^{bA}	41.85 ± 0.17^{bA}	32.71 ± 0.30^{cA}	27.19 ± 0.63^{dA}	46.16 ± 0.00^{bA}	27.78 ± 0.97^{bB}

Note: Capital letters indicate a significant difference between flushing and non-flushing and small letters indicate a significant difference part of plant at $p<0.05$.

Table 3: The total phenolic and flavonoid contents of C. sativa

Part of plant	TCC (mg/g extract)		Carotenoid (mg/g extract)	
	Flushing	Non-flushing	Flushing	Non-flushing
Fluorescence	0.71 ± 0.01^{dB}	0.77 ± 0.04^{dA}	0.24 ± 0.00^{bA}	0.27 ± 0.00^{bA}
Leaves	4.17 ± 0.05^{aA}	3.33 ± 0.17^{bA}	1.43 ± 0.02^{aA}	1.34 ± 0.12^{aA}
Twigs	1.49 ± 0.05^{cA}	1.31 ± 0.02^{cA}	0.39 ± 0.01^{bA}	0.40 ± 0.01^{bA}

Note: Capital letters indicate a significant difference between flushing and non-flushing and small letters indicate a significant difference part of plant at $p<0.05$.

4. Conclusion

The flushing process affects the chemical composition such as cannabinoids phytosterols, triterpenes, total phenolic, flavonoid, tannin and chlorophyll contents in each part of *C. sativa*. Moreover, their process influenced antioxidant activity. This research may be used to apply the cultivation of other plants to produce the bioactive compound in plants.

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