Journal of Harbin Engineering University
ISSN: 1006-7043

Vol 45 No. 3
March 2024

Quantitative Analysis of Cannabis Extracts at Different Alcohol Concentration and Extraction Time using HPLC Analysis

Montri Sangsuriyun[1],Sawinee Sangsuriyun[2*],Kowit Yodmongkol[3]

[1]Department of Industrial Engineering, Faculty of Engineering, Nakhon Phanom University Nong Yat, Mueang, Nakhon Phanom 48000, Thailand

[2*]Faculty of Science and Engineering, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus Chiang Khruea, Mueang, Sakon Nakhon 47000, Thailand

[3]Department of Electrical Technology, Faculty of Industrial Technology, Nakhon Phanom University, Nakhon Phanom 48000, Thailand

Abstract

This study examined the effects of varying alcohol concentrations—60%, 70%, 80%, 90%, and 95%—as well as extraction times—3, 7, and 30 days. High-performance liquid chromatography was used in a quantitative analysis in order to determine THC concentrations in the extracts. In Thailand, the law stipulates that THC concentrations in cannabis extracts used in the food, cosmetics, and herbal medicine industries, as well as in industries, must not exceed 0.2%. The analysis results showed that THC concentrations obtained via extraction for three and seven days with the majority of alcohol concentrations used in this study did not excess 0.2%. THC content of 0.2419 %w/w and CBD concentration of 0.0037 percent w/w were obtained via extraction using 95% alcohol, exceeding the 0.2% threshold. The THC and CBD concentrations from 30-day extraction were 0.2606, 0.2500, 0.2020, 0.2152, and 0.2137 %w/w and 0.0042, 0.0041, 0.0034, 0.0032, and 0.0030 %w/w, respectively. Therefore, the extracts need to be diluted with alcohol before use in order to keep the THC content under 0.2%. However, additional quantitative HPLC analysis is required. The contents of cannabis active substances were shown to be influenced by the extraction time and alcohol concentration. However, after a period of time, the contents of these substances remained constant. Therefore, the extraction period should not be longer than seven days, and the alcohol concentration should be between 60 and 90%.

Keywords:Alcohol concentration, cannabis extracts, extraction time, HPLC analysis.

1. Introduction

Cannabis or marijuana belongs to the family Cannabaceae. this perennial herb is indigenous to North America, Western Asia, and Europe and its scientific name is Cannabis [1-4]. Cannabis has different active substances based on the strain and cultivation conditions. In addition, there is the breeding of cannabis, which has sparked discussion on nomenclature and classification [5-7]. Furthermore, the comparative studies demonstrated that the content of cannabinoids in each strain of cannabis grown in each location varies. The primary substances in cannabis are cannabinoids. The two notable cannabinoids are THC and CBD. Like cannabis or marijuana (*Cannabis indica* Lam.), hem grows in Thailand and is a member of the Cannabaceae family. While the botanical features of hemp and cannabis are similar, their concentrations of active substances differ. According to the Narcotics Control Board's 2019 announcement on hemp characteristics, hemp is categorized as a subspecies of the cannabis plant, with THC content in leaves and inflorescences not to exceed 1.0% dry weight [8–9]. Due to its low THC content and long fibers, in Thailand, hemp has been promoted as a textile crop and its seeds have been used as food. According the report of the Medical Sciences Center 1, Chiang Mai, Department of Medical Sciences, hemp contains varying concentrations of active substances. The amounts of Δ9-THC and CBD in hemp ranged from 0.174 to 1.22% by weight and from 0.324 to 0.58% by weight, respectively. The environment, temperature, strain, age, growing and harvesting periods, and cultivation area with varying elevations all affect

the amounts of these active substances in hemp. In foreign countries, the seeds have been used as food [10]. The Food and Drug Administration has also supported the community enterprise groups with cannabis cultivation licenses in the use of the cannabis herb for medical and economic purposes. In order to advance the development of ingredients and cannabis products, there is also an aim to encourage, promote, and certify cannabis goods for the communities with cannabis cultivation licenses [11]. More than 60 phytocannabinoids and more than 460 active substances have been found in cannabis [12–13]. The chemical structures of these substances consist of the lipid, alkylresorcinol and terpenes. In cannabis, most cannabinoids are in the form of carboxylic acid, which has a low molecular weight. When exposed to light or heat, decarboxylation occurs, reducing the reaction of reduced form substances. The most important cannabinoids are Δ9-THC and CBD. Other important cannabinoids include Δ8-THC, cannabinol (CBN), cannabigerol (CBG) and terpenoid proteins [14-15]. Cannabis has been used for medicinal purposes to treat many diseases and disorders. However, at present there are a few research on the treatment of various diseases and conditions using cannabis. Therefore, it is still not possible to draw a clear conclusion regarding its effectiveness in treating certain diseases/symptoms. Cannabis is found to possibly effective against some diseases/conditions, depending on the disease and abnormality of each person, which are different. The Department of Medical Services, Ministry of Public Health has published recommendations for the use of medical cannabis for treatment. A study on the treatment of diseases and conditions (using active substances such as delta-9-tetrahydrocannabinol, cannabichomene, cannabidiol and cannabinol) [16] found that the condition that responded well to treatment with cannabis was anorexia. Some patients, especially those with HIV/AIDS or cancer, use cannabis to increase their appetite. Cannabis has been shown to be effective against diarrhea and chemotherapy-induced nausea and vomiting. It is also effective against cholestasis-associated pruritus. Additionally, THC is known to lower intraocular pressure, making it useful in treating glaucoma. However, its effects are temporary and often require alternative treatment. Cannabis is also effective for spasticity. Cannabis may benefit people with neurological conditions such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis (ALS) [17-19]. Symptoms with uncertain treatment results are anxiety and depression. CBD is found to be effective in relieving symptoms of anxiety and depression, potentially acting as a stress reliever without the psychoactive effects of THC. It also has a beneficial effect on asthma, cancer, headache, neuropathic pain and postoperative pain. It is often used to relieve chronic pain, including pain associated with conditions such as arthritis, multiple sclerosis, and myalgia. It is believed that THC and CBD have analgesic properties [20-22]. In this research, the extraction efficiency of cannabis using alcohol was studied [23-24]. Cannabis used in this research was from a community enterprise with a cannabis cultivation license. The extraction was performed using cannabis leaves at different alcohol concentrations of 60%, 70%, 80%, 90%, and 95% and extraction times of 3, 7, and 30 days. The CBD and THC contents in the extracts were determined by High Performance Liquid Chromatography (HPLC) analysis. The data of the amount of these active substances enables further development of cosmetic products or other products.

2. Materials and Methods

The cannabis leaves were weighed using a digital scale (Heavy Weighing Electronic Balance) as see shown in Fig. 1. Next, as illustrated in Fig. 2, the cannabis leaves were ground into a fine powder using a grinder. This experiment involved extraction over periods of 3, 7, and 30 days at varying alcohol concentrations of 60%, 70%, 80%, 90%, and 95%. Extraction was carried out by combining cannabis and alcohol at a 6:1 ratio as reported in [25–26]. Fifteen extract samples in all were prepared using the experimental design shown in Table 1. The extraction process involved soaking cannabis leaves in alcohol in a flask and shaking the flask twice a day for five minutes each time, as shown in Fig. 3.

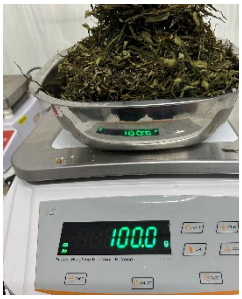

Fig. 1 Digital scale

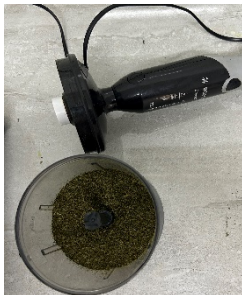

Fig. 2 Grinder

Fig. 3 Shaking of mixture

Table 1.Experimental design of cannabis extraction with alcohol

No.	Alcohol (Ak)	Extraction time(days)
1.	60%70% 80% 90% and 95%	3
2.	60%70% 80% 90%and 95%	7
3.	60%70% 80% 90% and 95%	30

This relationship can be represented mathematically by the following equation [27]:

$c_1V_1 = c_2V_2$ (1)

where

c_1 = initial concentration or molarity

V_1 = initial volume

c_2 = final concentration or molarity

V_2 = final volume

Analysis of Results

A comparative analysis was performed on 15 samples obtained by extraction of cannabis in alcohol at varying alcohol concentrations of 60%, 70%, 80%, 90%, and 95% over periods of 3, 7, and 30 days.

3. Results and Discussion

High-performance liquid chromatography (HPLC) analysis of CBD and THC contents in the extracts obtained by extraction of cannabis in alcohol at varying alcohol concentrations of 60%, 70%, 80%, 90%, and 95% over periods of 3, 7, and 30 days

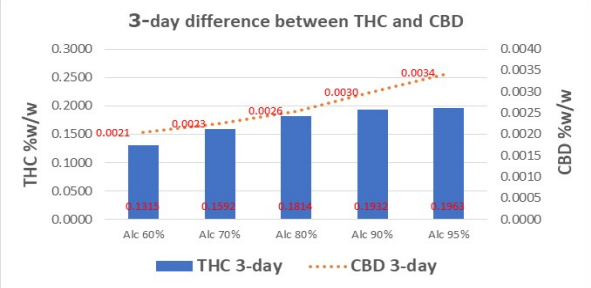

Fig. 4 Comparison of CBD and THC contents in 3-day extraction

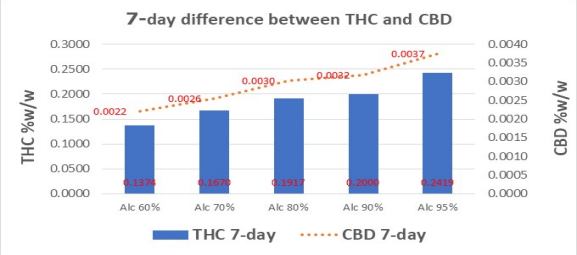

Fig. 5 Comparison of CBD and THC contents in 7-day extraction

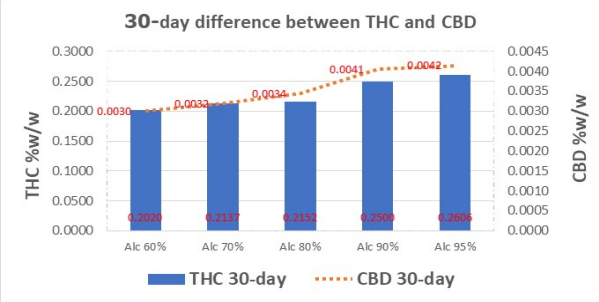

Fig. 6 Comparison of CBD and THC contents in 30-day extraction

High-performance liquid chromatography (HPLC) analysis of CBD and THC contents in the extracts obtained by extraction of cannabis at varying alcohol concentrations of 60%, 70%, 80%, 90%, and 95% over periods of 3 days are shown in Fig. 4. It was found that highest contents of THC (0.1932 and 0.963 %w/w) and CBD (0.0030 and 0.0034 %w/w) were obtained from the extraction with 90% and 95% alcohol. In summary, THC contents of the extracts obtained using 5 alcohol concentrations did not exceed 0.2% threshold. Therefore, permission is not required according to the drug law, which is in line with [28-29]. Extraction for a period of 7 days using 60%, 70%, 80%, and 90% alcohol resulted in low content of THC. The extracts obtained using 60% and 70% alcohol showed THC contents of 0.1374 and 0.1670 %w/w and CBD contents of 0.0022 and 0.0026 %w/w, respectively. The extracts obtained using 80% and 90% alcohol showed highest THC

contents of 0.1917 and 0.2000 %w/w and highest CBD contents of 0.0030 and 0.0032 %w/w, respectively. The extract obtained using 95% alcohol had the highest THC content of 0.2419 %w/w and CBD content of 0.0037 %w/w, which is higher than the 0.2% threshold. Therefore, the extract must be diluted by mixing with alcohol before use and additional test is required, which increase the cost. In addition, the test must be performed by government agencies with license issued by the Department of Science in accordance with the international laboratory standard ISO/IEC 17025. After the test, if the content of each active substance does not exceed 0.2%, the establishment must send the sample to the Provincial Public Health Office for obtaining a license before the substance can be used in the cosmetic industry both domestically and abroad. Alcohol is a commonly used solvent for extraction because it is easily available and commonly used in industry. However, if the amount of the active substance exceeds 0.2%, alcohol must be added to reduce the concentration of such substance [30]. In Italy, the latest law on industrial hemp (242/2016) sets the limit for the active substances from cannabis at 0.6%, especially for farmers. According to the Ministry of Interior, the raw materials in the market with substance at this level [31-32] can be processed into ingredients in cosmetics or used in other applications. In this study, the extracts obtained by soaking cannabis for 30 days at varying alcohol concentration of 60%, 70%, 80%, 90% and 95% contained more than 0.2% of THC, exceeding the limit set by law. However, the extracts obtained using 90% and 95% alcohol had the highest THC contents of 0.2500 and 0.2606 %w/w and CBD contents of 0.0041 and 0.0042 %w/w. The levels of THC and CBD in these extracts are suitable for the food industry, pharmaceutical industry and others, in accordance with [93]. While the extracts obtained using 60%, 70%, and 80% had THC contents of 0.2020, 0.2137, and 0.2152 % w/w and CBD contents of 0.030, 0.032, and 0.034 mg/g, respectively. The contents of cannabis active substances were shown to be influenced by the extraction time and alcohol concentration. However, after a period of time, the contents of these substances remained constant. The results also found that the extracts obtained using low alcohol concentrations had lower THC and CBD contents compared to the extracts obtained using high alcohol concentrations, which is consistent with [33]. The extraction of cannabis using ethyl alcohol and olive oil over extraction times of 0, 60, 120 and 180 minutes showed that the extracts obtained using ethyl alcohol had higher contents of active substances. It was also found that the longer extraction time and higher alcohol concentration resulted in a high amount of the active substance.

4. Conclusion

1) High-performance liquid chromatography (HPLC) analysis of CBD and THC contents in the extracts obtained by extraction of cannabis at varying alcohol concentrations of 60%, 70%, 80%, 90%, and 95% over periods of 3 days showed that THC contents of the extracts were 1.963, 1.932, 1.814, 1.592. and 1.315 mg/g, while the CBD contents were 0.034, 0.029, 0.026, 0.023, and 0.021 mg/g, respectively. All of these values do not exceed 0.2% THC limit and do not exceed legal requirements.

2) For 7-day extraction, it was found that 95% alcohol was effective in extracting active substances from cannabis, resulting in a THC concentration of 2.419 mg/g and a CBD concentration of 0.037 mg/g, which exceeds 0.2% limit. Therefore, the concentration of these active substances must be reduced before use by adding alcohol. However, additional quantitative HPLC analysis is required. When alcohol concentrations of 90%, 80%, 70%, and 60% were used, THC contents were 2.000, 1.917, 1.670, and 1.374 mg/g, and CBD contents were 0.032, 0.030, 0.026 and 0.022 mg/g, respectively. The concentration of THC was below 0.2% and does not exceed legal limits.

3) For 30-day extraction, it was found that when 95%, 90%, 80%, 70% and 60% alcohol were used, the THC contents were 2.606, 2.500, 2.020, 2.152 and 2.137 mg/g. while the CBD contents were.042, 0.041, 0.034, 0.032 and 0.030 mg/g, respectively. Therefore, the extracts need to be diluted with alcohol before use in order to keep the THC content under 0.2%. However, additional quantitative HPLC analysis is required. If the

content of each active substance does not exceed 0.2%, the license is not required under the drug laws and the substances can be processed into ingredients in cosmetics or used in other applications. The contents of cannabis active substances were shown to be influenced by the extraction time and alcohol concentration. However, after a period of time, the contents of these substances remained constant. A high alcohol concentration can reduce the time needed for extraction However, additional confirmation test is required.

5. Recommendations

1) During HPLC analysis, results should be verified by additional THC and CBD extractions from cannabis-alcohol mixtures at varying alcohol concentration of 60%, 70%, 80%, 90%, and 95%.
2) In the extraction of Thai Stick cannabis, HPLC analysis may result in errors or discrepancies in THC and CBD contents compared to other strains.
3) Shaking of cannabis-alcohol mixture twice a day, in the morning and evening for 5 minutes, may lead to ineffective extraction of the active substances. It is best to use an automatic shaker.

Acknowledgements

This research was funded by the Science, Research and Innovation Program 2023, with the research proposal code 66A134000012. The researcher would like to express my gratitude to the Smart Materials Group, Department of Industrial Engineering and Management, Faculty of Engineering, Nakhon Phanom University and Kasetsart University Laboratory, Chaloem Phrakiat Campus, Sakon Nakhon Province.

References

[1] S. Chandra, H. Lata, and I. A. Khan, and M. A. ElSohly, “Cannabis sativa L.: Botany and horticulture,” Cannabis sativa L. - Botany and Biotechnology, pp. 79–100, May 2017.

[2] M. Pillay, and S. T. Kenny, “Structural organization of the nuclear ribosomal RNA genes in Cannabis and Humulus (Cannabaceae),” Plant Systematics and Evolution, vol. 258, pp. 97-105, March 2006.

[3] J. M. McPartland, “Cannabis systematics at the levels of family, genus, and species,” Cannabis and cannabinoid research, vol. 3(1), pp. 203-212, October 2018.

[4] A. Javaid, I. H. Khan, and M. F. Ferdosi, “Bioactive constituents of wild Cannabis sativa roots from Pakistan,” Pakistan Journal of Weed Science Research, vol. 27(3), pp. 359, April 2021.

[5] J. M. McPartland, and E. Small, “A classification of endangered high-THC cannabis (Cannabis sativa subsp. indica) domesticates and their wild relatives,” PhytoKeys, vol. 144, pp. 81, April 2020.

[6] A. Hazekamp, and J. T. Fischedick, “Cannabis - from cultivar to chemovar,”Drug testing and analysis, vol. 4(7-8), pp. 660-667. February 2012.

[7] J. Bosse, “Before the High Court: the legal systematics of Cannabis,” Griffith Law Review, vol. 29(2), pp. 302-329. May 2020.

[8] A. Pollio, “The name of Cannabis: a short guide for nonbotanists,” Cannabis and cannabinoid research, vol. 1(1), pp. 234-238, October 2016.

[9] C. E. Turner, M. A. Elsohly, P. C. Cheng, and G. Lewis, “Constituents of Cannabis sativa L., XIV: Intrinsic problems in classifying cannabis based on a single cannabinoid analysis,” Journal of Natural Products, vol. 42(3), pp. 317-319. May 1979.

[10] P. Tipparat, “Cannabis: Knowledge and examination of active substances,” Retrieved 10 February 2020, from https:// www.oncb.go.th/ncsmi/ cannabis4/ พืชกัญชาความรู้ทั่วไปและการตรวจสอบสารสำคัญ

[11] Y. Pattanawong, P. Angtrakul, W. Asawakitwiri. W. Yungthong, A. Asawamekon, and K. Netthip, “Development of health product regulatory system for promotion of research and innovation,” Food and Drug Journal, vol. 25(2), pp. 18-28, August 2018.

[12] R. P. Latta, and B. J. Eaton, “Seasonal fluctuations in cannabinoid content of Kansas marijuana,” Economic Botany, vol. 29(2), pp. 153-163, April 1975.

[13] C. Rong, Y. Lee, N. E. Carmona, D. S. Cha, R. M. Ragguett, J. D. Rosenblat, and R. S. McIntyre, “Cannabidiol in medical marijuana: research vistas and potential opportunities,”

Pharmacological Research, vol. 121, pp. 213-218, July 2017.

[14] B. Stella, F. Baratta, C. Della Pepa, S. Arpicco, D. Gastaldi, and F. Dosio, "Cannabinoid formulations and delivery systems: Current and future options to treat pain," Drugs, vol. 81, pp. 1513-1557, September 2021.

[15] L. D. Carreira, F. C. Matias, and M. G. Campos, "Clinical data on canabinoids: translational research in the treatment of autism spectrum disorders," Biomedicines, vol. 10(4), pp. 796, March 2022.

[16] G. Hsu, Ö. Koçak, and B. Kovács, "Co-opt or coexist? A study of medical cannabis dispensaries' identity-based responses to recreational-use legalization in Colorado and Washington," Organization Science, vol. 29(1), pp. 172-190, January 2018.

[17] M. E. Badowski, and P. K. Yanful, "Dronabinol oral solution in the management of anorexia and weight loss in AIDS and cancer," Therapeutics and clinical risk management, vol. 6(14), pp. 643-651, April 2018.

[18] K. A. Belendiuk, L. L. Baldini, and M. O. Bonn-Miller, "Narrative review of the safety and efficacy of marijuana for the treatment of commonly state-approved medical and psychiatric disorders," Addiction Science & Clinical Practice, vol. 10(1), pp. 1-10, April 2015.

[19] M. E. Gerich, R. W. Isfort, B. Brimhall, and C. A. Siegel, "Medical marijuana for digestive disorders: high time to prescribe?," Official journal of the American College of Gastroenterology| ACG, vol. 110(2), pp. 208-214, February 2015.

[20] J. D. Kosiba, S. A. Maisto, and J. W. Ditre, "Patient-reported use of medical cannabis for pain, anxiety, and depression symptoms: Systematic review and meta-analysis," Social science & medicine, vol. 233, pp. 181-192, July 2019.

[21] C. Noel, "Evidence for the use of "medical marijuana" in psychiatric and neurologic disorders," Mental Health Clinician, vol. 7(1), pp. 29-38, January 2017.

[22] J. Turna, B. Patterson, and M. Van Ameringen, "Is cannabis treatment for anxiety, mood, and related disorders ready for prime time?," Depression and anxiety, vol. 34(11), pp. 1006-1017, November 2017.

[23] P. Tipparat, "Cannabis: Knowledge and examination of active substances," Retrieved 10 February 2020, from https:// www.oncb.go.th/ncsmi/ cannabis4/ พืชกัญชาความรู้ทั่วไปและการตรวจสอบสารสำคัญ

[24] Announcement of the Ministry of Public Health regarding the list of narcotics under category 5 (2020), Royal Gazette, Volume 137, Special Section 290 D (Date 14 December 2020, pp. 33-34.

[25] G. Appendino, S. Gibbons, A. Giana, A. Pagani, G. Grassi, M. Stavri, and M. M. Rahman, "Antibacterial cannabinoids from Cannabis sativa: A structure− activity study," Journal of natural products, vol. 71(8), pp. 1427-1430, August 2008.

[26] C. Monton, N. Chankana, S. Leelawat, J. Suksaeree, and T. Songsak, "Optimization of supercritical carbon dioxide fluid extraction of seized cannabis and self-emulsifying drug delivery system for enhancing the dissolution of cannabis extract," The Journal of Supercritical Fluids, vol. 179, pp. 105423, January 2022.

[27] B. Bassa, and R. Uppu, "Modification of the Basic Dilution Equation for the Programming of Serial Dilutions, ChemRxiv, April 2022.

[28] M. Dei Cas, E. Casagni, A. Saccardo, S. Arnoldi, C. Young, S. Scotti, and G. Roda, "The Italian panorama of cannabis light preparation: determination of cannabinoids by LC-UV," Forensic science international, vol. 307, pp. 110113, February 2020.

[29] S. Sgrò, B. Lavezzi, C. Caprari, M. Polito, M. D'Elia, G. Lago, and E. N. Ferri, "Delta9-THC determination by the EU official method: Evaluation of measurement uncertainty and compliance assessment of hemp samples," Analytical and bioanalytical chemistry, vol. 413, pp. 3399-3410, March 2021.

[30] E. P. Baron, "Medicinal properties of cannabinoids, terpenes, and flavonoids in cannabis, and benefits in migraine, headache, and pain: an update on current evidence and cannabis science," Headache: The Journal of Head and Face Pain, vol. 58(7), pp. 1139-1186, July 2018.

[31] B. Stella, F. Baratta, C. Della Pepa, S. Arpicco, D. Gastaldi, and F. Dosio, "Cannabinoid formulations and delivery systems: Current and future options to treat pain," Drugs, vol. 81, pp. 1513-1557, September 2021.

[32] Department of Medical Services, Ministry of Public Health, "Guidance on the use of medical marijuana, "Revision No. 1/2019, Retrieved 10 February 2020, from https://www.chiangmaihealth.go.th/cmpho_web/document/190609156008311353.

[33] C. Citti, U. M. Battisti, D. Braghiroli, G. Ciccarella, M. Schmid, M. A.Vandelli, and G. Cannazza, "A metabolomic approach applied to a liquid chromatography coupled to high-resolution tandem mass spectrometry method (HPLC-ESI-HRMS/MS): towards the comprehensive evaluation of the chemical composition of Cannabis medicinal extracts," Phytochemical Analysis, vol. 29(2), pp. 144-155, March 2018.