ORIGINAL ARTICLE

Assessment of pharmacognostic specification of *Cannabis sativa* leaves in Thailand

Kanittha Nakkliang,
Chitlada Areesantichai,
Kanchana Rungsihirunrat

Department of Public Health Sciences, College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand

J. Adv. Pharm. Technol. Res.

ABSTRACT

Lack of quality control can affect the safety, efficacy, and acceptability of herbal products that may lead to health problems. *Cannabis sativa* L. (Cannabaceae) has been widely used as an ethnomedicinal practice for its medicinal values. This study aims to establish pharmacognostic specifications of *C. sativa* as per standard procedures. Macroscopic-microscopic characteristics, physicochemical parameters, thin-layer chromatography (TLC) fingerprinting, and phytochemical screening of *C. sativa* leaves collected from various locations throughout Thailand were investigated. Leaves are palmate consists of seven leaflets with green color, margin is serrate with acuminate apex. Anomocytic stomata were found in the upper epidermis while unicellular and glandular trichomes with cystolith were found in the lower epidermis and the epidermis layer covered with cuticle. The physicochemical analysis revealed that the loss on drying (4.068 ± 0.084 %w/w) was within acceptable limits, total ash (14.360 ± 0.084%w/w), acid insoluble ash (2.726 ± 0.080%w/w), ethanol-soluble extractive (11.101 ± 0.223%w/w), water-soluble extractive (23.038 ± 0.306%w/w), and water content (7.523 ± 0.524%w/w). TLC fingerprint showed nine spots with Rf value 0.14, 019, 0.23, 0.29, 0.32, 0.45, 0.58, 0.70, and 0.76. Phytochemical screening of Cannabis leaves indicated the presence of phenolic compounds, flavonoids, alkaloids, diterpenes, triterpenes, and steroids. This study provided referential data for the accurate plant identity, and establishment of cannabis leaves monograph in Thailand.

Key words: Cannabis, pharmacognostic specification, physicochemical, phytochemical screening

INTRODUCTION

The demand for herbal medicines is growing more and more due to their long historical use and fewer side effects.[1,2] *Cannabis sativa* L is a genus of annual, dioecious, and flowering plants in the family Cannabaceae and indigenous from Central Asia, Asia, and Europe.[3] Cannabis has some medicinal properties, for example, reducing nausea and vomiting during chemotherapy, chronic pain, muscle spasms, analgesic, intoxicant, stomachic, and sedative.[4-7] In 2019, Thailand, is the first country in Southeast Asia that allow cannabis to be used for medical purposes and research.[8] As a result, this study is the first report for the standardization of C. sativa dry leaves in Thailand.

Address for correspondence:
Dr. Kanchana Rungsihirunrat,
College of Public Health Sciences, Chulalongkorn University,
Bangkok 10330, Thailand.
E-mail: kanchana.r@chula.ac.th

Submitted: 02-Apr-2022 **Revised:** 24-May-2022
Accepted: 01-Jun-2022 **Published:** 05-Jul-2022

Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/japtr.japtr_96_22

How to cite this article: Nakkliang K, Areesantichai C, Rungsihirunrat K. Assessment of pharmacognostic specification of *Cannabis sativa* leaves in Thailand. J Adv Pharm Technol Res 2022;13:226-31.

As *C. sativa* has been an important source of medicinal substances, it is necessary to develop standardization to determine the quality, safety, and efficacy of cannabis materials. Herbal medicines are natural products, their phytochemical constituents depend on many factors[9-11] and these variations could impact its efficacy profile. Recently, standard methods for quality control of herbal medicines were established. There are various parameters that should be considered; macroscopic-microscopic evaluation, physicochemical evaluation, phytochemical evaluation, toxicity, and biological activity.[12] Lack of quality control can affect the safety, efficacy, and acceptability of herbal products that may lead to health problems. Therefore, quality control of herbal products is essential. Although in Thai herbal pharmacopeia 2021, the monograph of the dried female flower of *C. sativa* have been prepared, but still lacked pharmacognostic specification of *C. sativa* leaves. Therefore, this study was designed to evaluate the pharmacognostic specification of *C. sativa* leaves for its authenticity, purity, and quality control of plant material for Thai traditional medicine remedies.

MATERIALS AND METHODS

Plant samples

The *C. sativa* leaves were kindly received from the Drug Dependence Research Center, College of Public Health Sciences, Chulalongkorn University. Twelve samples of *C. sativa* leaves were collected during August–December 2020 from various locations throughout Thailand. Plant samples were authenticated and deposited in voucher specimens at the College of Public Health Sciences, Chulalongkorn University, Thailand. Plant samples were washed and oven drying at 45°C–50°C.

Determination of standardization parameters

The standardization parameters including macroscopic-microscopic, physicochemical, thin-layer chromatography (TLC) fingerprinting, and phytochemical analysis were investigated according to the World Health Organization (WHO) guidelines.[14]

Macroscopic examination

Morphological and organoleptic characteristics of *C. sativa* fresh mature leaves were analyzed by visual inspection and recorded.

Microscopic examination

The transverse section of the midrib and lamina of *C. sativa* leaves was examined under a microscope at × 20 and × 40. The fresh mature leaves were cut in parallel including the midrib and lamina into pieces as thin as possible using sharp razor blades by free hand sectioning, cleared with chloralhydrate, and transferred onto a slide in glycerin water for microscopic examination. Photomicrographs of different magnifications were taken and recorded with Axio Vision 4.0 V4.6.3.0 software (Carl Zeiss Imaging Solutions, Munich, Germany).

Powder drug examination

Shade-dried C. sativa material was grounded into a fine powder, mounted with water, and examined under the microscope.[15]

Physicochemical determination

Physicochemical determination was investigated according to the WHO guidelines.[14] All physicochemical parameters were performed in triplicate. The data were exhibited by mean ± standard deviation.

Determination of loss on drying

Weighted 3 g of the *C. sativa* dried powder in a crucible and then heated at 105°C until constant weight. The percentage of loss on drying was calculated.

Determination of water content (Azeotropic Distillation Method)

Weighted 50 g of *C. sativa* dried powder and placed it into the round bottom flask. Water-saturated toluene (200 ml) was added and boiled by using azeotropic apparatus. After completely distilled, allowed the receiving tube to be cooled in room temperature. Observed the water-toluene separated layers and calculated water content as the percentage of dry weight.

Determination of total ash

Placed 3 g of the *C. sativa* dried powder in a crucible. The sample was ignited at 500°C until white ash was obtained. The percentage of total ash was calculated.

Determination of acid-insoluble ash

Added 50 ml of 2N HCL to the total ash, and the mixture was boiled gently for 5 min. The insoluble matter was filtrated (ashless filter paper No. 40), then transferred to the original crucible. After drying, ignited the crucible until obtain constant weight. The residue was allowed to cool in desiccators and weighed. Calculated the percentage.

Determination of ethanol and water extractive value

Added 100 ml of absolute ethanol into 5 g of *C. sativa* dried power in a closed conical flask in shaking bath for 6 h and let stand for 18 h. The extract was filtered, evaporated to dryness, and then heated until obtained constant weight. For water-soluble extraction, use water in place of ethanol.

Determination of volatile oil content (Clevenger distillation method)

Added water (200 ml) into 100 g *C. sativa* dried powder in the round bottom flask. After volatile oil completely separated from water, measured, and calculated volatile oil volume.

Thin layer chromatography fingerprint

Ethanol extract (20 mg) was dissolved in 1 ml methanol and applied (3 µl) onto the silica gel 60 F254 TLC plate

using A Linomat IV. The TLC plate was developed with a solvent system; hexane: ethyl acetate: acetic acid (4:1:0.5) and observed spots on the plate under white light, short-wavelength (254 nm), and ultraviolet light and sprayed the plate with 0.5% fast blue B salt. Rf value was calculated[16,17] following this formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Phytochemical screening

The phytochemical screening was performed based on the standard method[18] to detect the presence of phenolics (ferric chloride test), alkaloids (Dragendorff's and Wagner's test), flavonoids (alkaline reagent test), triterpenes and steroids (Salkowski and Liebermann-Burchard test), diterpenes (copper acetate), and saponin (foam test).

RESULTS

C. sativa leaves were collected from 12 different geographic regions throughout Thailand, as shown in Table 1.

Macroscopic examination

Morphologically, the fresh mature leaf of *C. sativa* is compound, palmate shaped, 7–9 linear-lanceolate leaflet blades, serrate margin with acuminate apex, alternate or opposite in arrangement. The upper (adaxial) surfaces are dark green, while the lower (abaxial) are pale green with rough surfaces. Leaves are 0.2–2 cm wide, 3–15 cm long, and 2–-7 cm petiole [Figure 1] and bitter test.

Microscopic examination

The upper epidermis surface revealed rectangular cells with striations, anomocytic stomata, palisade, and cicatrix. Trichomes consist of glandular trichome and unicellular trichome with cystolith found in the lower epidermis [Figure 2].

The transverse section of the midrib and lamina of *C. sativa* leaf [Figure 3] showed the upper and lower epidermis surface is covered by a single layer of the epidermis. The epidermis is undulating, with unicellular nonglandular trichome and glandular trichomes. The midrib was composed of collenchyma layer cell underneath the upper and lower epidermal, parenchyma containing rosette aggregate crystal. The mesophyll showed of distinct palisade layer and spongy parenchyma. The vascular bundle was surrounded by sclerenchyma tissue.

The powder microscopic examination of *C. sativa* leaves indicated the presence of anomocytic stoma, palisade, parenchymacell containing rosette aggregate crystal, spiral vessels, unicellular trichomes, cystolithic, and fiber, as shown in Figure 4.

Table 1: The locations of 12 *Cannabis sativa* samples collected throughout Thailand

Number	Sources of *Cannabis sativa* leaves collection	Voucher specimen (number)	Locations
1	CM-1	CSL01/2020	Northern
2	CM-2	CSL02/2020	Northern
3	CM-3	CSL03/2020	Northern
4	LP-1	CSL04/2020	Northern
5	PH-1	CSL05/2020	Northern
6	SK-1	CSL06/2020	Northeastern
7	SK-2	CSL07/2020	Northeastern
8	SK-3	CSL08/2020	Northeastern
9	MD-1	CSL09/2020	Northeastern
10	NK-1	CSL10/2020	Northeastern
11	SR-1	CSL11/2020	Central
12	SR-2	CSL12/2020	Central

CM: Chiang Mai, LP: Lampang, PH: Phrae, SK: Sakhonnakorn, MD: Mukdahan, NK: Nakhonrajchasima, SR: Saraburi

Physicochemical parameters

The physicochemical parameters of *C. sativa* leaves collected from the different geographic regions were found to be in an acceptable range, as summarized in Table 2.

Thin-layer chromatography fingerprinting

TLC fingerprints of this extract are shown in Figure 5. TLC pattern of ethanolic extract indicated nine spots with Rf value 0.14, 019, 0.23, 0.29, 0.32, 0.45, 0.58, 0.70, and 0.76 using the solvent system *n*-hexane, ethyl acetate, acetic acid (4:1:0.5) with 0.5% Fast blue B Salt as staining reagent.

Phytochemical screening

Phytochemical screening of *C. sativa* ethanol extract showed the presence of alkaloids, flavonoids, phenolics, steroids, triterpenes, and diterpenes. However, saponin was not detected [Table 3].

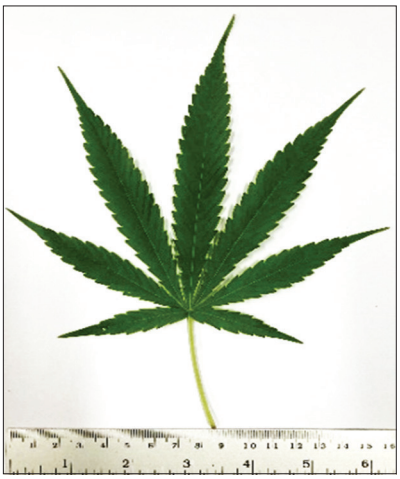

Figure 1: Leaf of *Cannabis sativa*

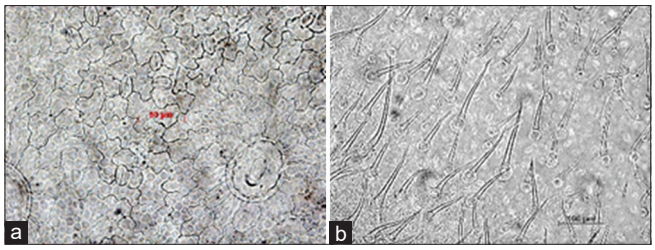

Figure 2: Upper epidermis (a), lower epidermis (b)

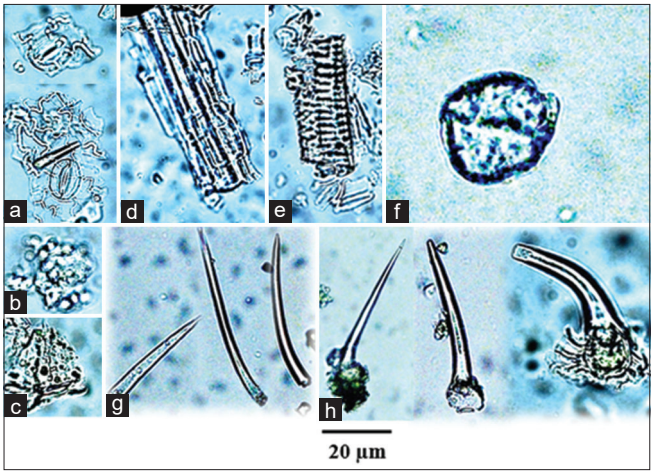

Figure 4: Powder microscopy of *Cannabis sativa* leaf; anomocytic stoma (a), palisade cell (b), parenchyma cell containing rosette aggregate crystal (c), fiber (d), spiral vessels (e), cystolith (f) unicellular trichomes (g), unicellular trichomes with cystolith (h)

DISCUSSION

Establishing pharmacognostic standards is necessary for the evaluation of medicinal plants. According to the WHO, the organoleptic and microscopic characteristics are the starting step toward identification and evaluation of purity.[14] Microscopic characterization is one of the diagnostic features for the identification of medicinal plants, broke of crude drugs or small fragments, and detection of adulterants, substituents, and authentic plants. Epidermal characteristics and stomata are also wildly used in identification at genus and species levels. The powdered drug was assessed for its structural cell and physicochemical analysis.[19]

Loss on drying measures the amount of volatile oil and water containing in the plant material. The loss on drying of *C. sativa* leaves powder was presented 4.068 ± 0.084%w/w. To prevent the decomposition of crude drugs, the water content of the crude drugs should be minimized ranging from 10% to 20% which is an ideal range for minimum bacterial and fungal growth.[20] The water content was measured using the azeotropic distillation method, the result showed that it should not be higher than 7.954 ± 0.324%w/w. Total ash values of crud drug give the concept of inorganic matter and other impurities; phosphates, carbonates, silica, and silicates.[21,22] Ash values of *C. Sativa* powdered leaves were

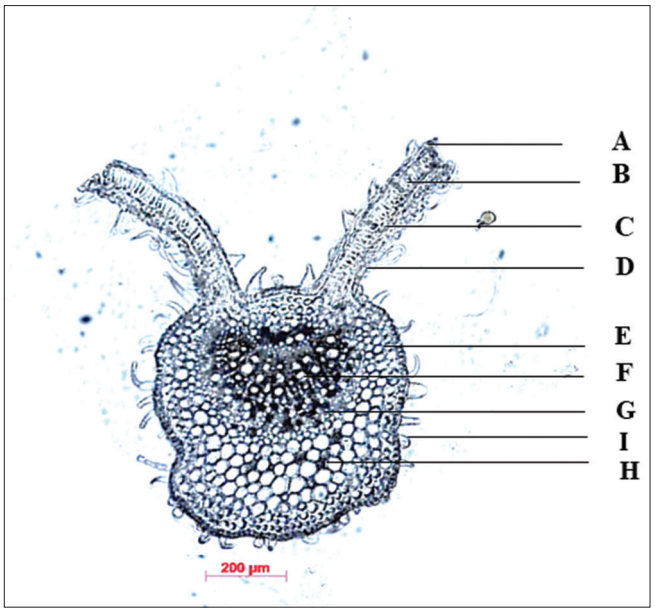

Figure 3: Transverse section of *Cannabis sativa* leaves at × 20 (A) upper epidermis, (B) palisade cell, (C) spongy cell, (D) lower epidermis, (E) collenchyma, (F) Vascular bundle (phloem and xylem tissue), (G) Sclerenchyma tissue, (H) Parenchyma containing rosette aggregate crystal, (I) Trichome (unicellular, glandular)

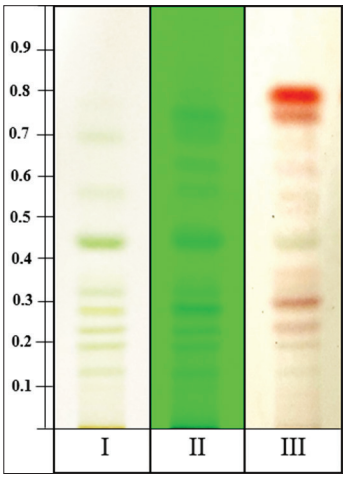

Figure 5: TLC fingerprint of *Cannabis sativa* leaf ethanolic extract. I: Detection under white light, II: Detection under UV 254 nm, III: Detection with 0.5% fast blue B salt staining reagent. TLC: thin-layer chromatography

found to be 14.360 ± 0.165%w/w, while acid insoluble ash was found to be 2.726 ± 0.080% w/w which indicates that crude drug is low contamination. The different extractive values were indicated the chemical composition present in crude drug and solubility in a specific solvent.[23] The result of *C. sativa* leaves powdered showed that the water extractive value (23.038 ± 0.306) was higher than the ethanol extractive values (11.101 ± 0.223%w/w), indicating the presence of water-soluble compounds contained in plant materials and their different solubility property in different solvents. In this study, the volatile oil content was not detected from

Table 2: Physicochemical parameters of cannabis leaves

Physicochemical parameter	Value (% w/w)
Loss on drying	4.068±0.084
Water content	7.954±0.324
Total ash	14.360±0.165
Acid insoluble ash	2.726±0.080
Water-soluble extractive value	23.038±0.306
Ethanol-soluble extractive value	11.101±0.223
Volatile oil content	Not detected

The value was shown as mean ± SD. SD: Standard deviation

Table 3: Phytochemical screening of *Cannabis sativa* leaves

Phytoconstituents	Phytochemical test	Ethanolic extract
Alkaloids	Dragendroff 's test	+
	Wagner's test	+
Flavonoids	Alkaline test	+
Phenolics	Ferric chloride test	+
	Shinoda test	+
Steroids	Salkowski test	+
Triterpenes	Liebermann–Burchard test	+
Diterpenes	Copper acetate test	+
Saponin	Foam test	–

All sample was done in triplicate. +: Present, –: Absent

dry leave powder with hydro distillation method when compared to previously studied using fresh leaves, this may be happened due to the volatility of essential oil components which may be decreased on the drying processing. Based on the taxonomic approach, plants in the same genus will contain the same chemical compounds. However, the yield and quality of essential oil are affected by a variety of environmental factors, including climatic conditions, geographical cultivation, and the extraction process.[24]

The phytochemical screening is a qualitative determination of the class of compounds contained in the plant. Phenolics, flavonoids, steroids, triterpenoids, diterpenes, and alkaloids were presented in *C. sativa* leaves which may be responsible for some medicinal properties. However, saponins were undetectable in the crude ethanolic extract of *C. sativa* leaves. The findings in accordance with the previous study of *C. sativa* leaves cultivars from China and India.[16,25] Phytochemical examinations are helpful in quality evaluation and drug discovery. The parameters studied in the present work may be successfully used for quality evaluation and could be applied as a standard reference, quality control, monograph preparation, and assurance of crude drug.

CONCLUSION

The characteristics of *C. sativa* leave according to macroscopic-microscopic examination, physicochemical parameter, TLC fingerprinting and phytochemical screening can be served as a standard reference for traditional practices and used to characterize the identity and quality of medicinal plant materials.

Acknowledgments

This work was supported by the Students Research Support of Chulalongkorn University Scholarship. The authors appreciate all staff members of the College of Public Health Sciences, Chulalongkorn University, Thailand, for their assistance and instrument support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. World Health Organization. Traditional Medicine Strategy. Geneva, Switzerland: WHO; 2002.
2. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014;4:177.
3. Kuddus M, Ginawi IA, Al-Hazimi A. *Cannabis sativa*: An ancient wild edible plant of India. Emirates J Food Agric 2013;25:736-45.
4. Whiting PF, Wolff RF, Deshpande S, Di Nisio M, Duffy S, Hernandez AV, *et al.* Cannabinoids for medical use: A systematic review and meta-analysis. JAMA 2015;313:2456-73.
5. Goutopoulos A, Makriyannis A. From cannabis to cannabinergics: New therapeutic opportunities. Pharmacol Ther 2002;95:103-17.
6. Russo E, Guy GW. A tale of two cannabinoids: The therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. Med Hypotheses 2006;66:234-46.
7. Pagano C, Navarra G, Coppola L, Avilia G, Bifulco M, Laezza C. Cannabinoids: Therapeutic use in clinical practice. Int J Mol Sci 2022;23:3344.
8. Notification of the Ministry of Public Health Re: Prescribing Formulas of Narcotics of Category V that are Consumable for Treatment of Disease or Research Study. Government Gazette, Vol. 136, Special Issue 199. Dated 8th August, B.E. 2562; 2019. p. 11.
9. Kokate CK, Poruhit AP, Gokhale SB. Phamacognosy Constituent. 2nd ed. New Delhi: Valklabh Prakashan; 2004. p. 466-70.
10. Park JH. Evidence-based herbal medicine in efficacy and safety assessments. Orient Pharm Exp Med 2008;8:103-10.
11. Li Y, Kong D, Fu Y, Sussman MR, Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem 2020;148:80-9.
12. Bijauliya RK, Alok S, Chanchal DK, Kumar M. A comprehensive review on standardization of herbal drugs. IJPSR 2017;8:3663-77.
13. Department of Medical Sciences. Thai Herbal Pharmacopeia 2021. Bangkok: Department of Medical Sciences; 2021.
14. World Health Organization. Quality Control Methods for Herbal Materials. Geneva: WHO; 2011.
15. Evans WC. Trease and Evans Pharmacognosy. 15th edition. Delhi, India: Elsevier Publisher; 2009.
16. Kumari P, Kumari C, Singh PS. Phytochemical screening of selected

medicinal plants for secondary metabolites. Int J Life Sci Sci Res 2017;3:1151-7.

17. Joshi BC, Panthri N, Prasad N, Virk JK. Pharmacognostic and physico-chemical investigation of *Barleria cristata* Linn. (Leaf) for quality control assessment. PBR 2021;7:191-200.
18. Alebiosu CO, Yusuf AJ. Phytochemical screening, thin-layer chromatographic studies and UV analysis of extracts of *Citrullus lanatus*. J Pharm Chem Biol Sci 2015;3:214-20.
19. Adom E, Ekuadzi E, GyasiIsaac EB, Amponsah IK. Pharmacognostic evaluation of three *Apocynaceae* plants that share the same West African local names. Phytomed Plus 2022;2:100157.
20. Chumbhale DS, Upasani CD. Pharmacognostic standardization of stems of *Thespesia lampas* (Cav.) Dalz & Gibs. Asian Pac J Trop Biomed 2012;2:357-63.
21. Upadhyay P, Joshi BC, Sundriyal A, Minky M. Pharmacognostic standardization and physicochemical evaluation of *Caesalpinia crista* L. root for quality control assessment. J Nat Sci Med 2019;2:135-40.
22. Pandiyan R, Ilango K. Pharmacognostical, physicochemical and phytochemical evaluation of *Huberantha senjiana* (*Annonaceae*) leaf: An endemic tree of Gingee Hills Tamil Nadu India. J Pharm Pharmacogn Res 2022;10:158-72.
23. Hanani E, Soewandi HS, Hayati RN. Pharmacognostical and preliminary phytochemical evaluation of *Cordia sebestena* L. Pharmacogn J 2019;11:1100-5.
24. Naz S, Hanif MA, Bhatti HN, Ansari TM. Impact of supercritical fluid extraction and traditional distillation on the isolation of aromatic compounds from *Cannabis indica* and *Cannabis sativa*. J Essent Oil Bear Plants 2017;20:175-84.
25. Ahmed M, Ji M, Qin P, Gu Z, Liu Y, Sikandar A, *et al*. Phytochemical screening, total phenolic and flavonoids contents and antioxidant activities of *Citrullus colocynthis* L. and *Cannabis sativa* L. Appl Ecol Environ Res 2019;17:6961-79.