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Research Article

# Chemical Profile of Crude Extracts from Cannabis sativa L. dried stems with Gas Chromatography Techniques

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**Abstract**

The stems of *Cannabis Indica* were allowed to be used. The *Cannabis sativa L*. dried stems were subjected to extraction of chemical constituents. The extraction of cannabis stems performed by maceration process (solvent: hexane, 95%Ethanol and 80%methanol and distilled water; for 7days) and solvent evaporation. The GC-MS analysis were revealed the phytochemicals composition of the crude. Among the data analysis of chemical compounds were presented the pharmacological effects as antioxidant, antimicrobial and anti-inflammatory, respectively. The volatile compounds and medically used compounds were determined. The experimental results were shown the usable of cannabis stems. The selection of the suitable solvents and the properties of essential substances, were determined.

**Keywords;** GC-MS, Cannabis, Chemical Constituents

## 1. Introduction

Cannabis sativa L. or marijuana was a plant in the warmer climate areas such as Asia, South America and the Middle East. The marijuana was used application for over four thousand years, such as for feeding people or animals. It was used as the narcotic for relaxation and the fibers for apply equipment such as ropes or clothing. The medical purposes, have been reports in the history. The marijuana was used as the Medical, that widespread on many continents. The medical application began to be systematically reported in Europe and America in the 19th century. The chemical constituents have been discovered in cannabis, which the main substance was Delta-9-tetrahydrocannabinol (THC). The previous research conducted to study psychoactive substances from leaves and i nflorescences. The THC entered to the brain, anchored on cannabinoid receptors cause for euphoria, anxiety and some people had altered environmental perceptions. The second, the most important substance was Cannabidiol (CBD), which has a less psychoactive effect. The discovery of this important substance intensified marijuana control in 1937. Cannabis was used as non-toxic doses affects the mind (psychological effect) such as feeling relaxed, happy, time passes slowly and grows more food and affects the body. (McConachie et al., 2019).

The U.S. government passed the marijuana tax act law of Federal Bureau of Narcotics. In 1941, the marijuana has been withdrawn from the American pharmacopoeia. The United Nations included the marijuana in a Single convention on narcotic drugs in 1961, it contained an important substance: not for sell or produce drugs or substances that act on psychotropic, excepted for research purposes on the medical utilization (Thipra, 2009) Gas chromatography mass spectrometry (GC-MS) was a technique for screening the type of constituents, a common technique that combines two advantages: the first, to separate the mixtures that become easily vaporized into single elements with a high separation capacity, and on the other hand, each substance that has been isolated was measured in a mass spectrometry method. That is specific to measurements, sensitive to high measurement and provides information. Mass spectrum of substances can be used to effectively prove the substance's identity. It was the connection of two techniques: gas chromatography, which was a separation technique, and mass spectrometry, a measurement technique used to prove identity and quantify substances. The combination of these two techniques produces several positive effects (Buranaosot, 2016)

Cannabis stems composed of a high fiber, which was a source of natural fiber and is used as animal feed. Hemp bast fibers as antibacterial and used for the manufacture of an antibacterial agent or functionalized textiles, has been reported. These compounds possess known as antibacterial properties, found to contain cannabinoids (2% of the total metabolite extract). The hemp powder shown the antibacterial properties against Escherichia coli. and a higher lignin content than the bast fibers, the antibacterial property may be linked to lignin-related compounds such phenolic compounds, as well as alkaloids and cannabinoids (Tayyab & Shahwar, 2015)

The previous research of cannabis plants revealed the pharmacological active substance. The objectives of this study, to compare the chemical compositions of cannabis stems based on different solvent extraction. The active compounds were analyzed by gas chromatography techniques to provide information on the further application.

## 2. Material and Methods

### 2.1 Sample Preparation

Cannabis stems were obtained from greenhouses demonstrate medical cannabis cultivation of RMUTI-SKC. The sample was cleaned and dried in a solar-powered drying plant and coarsely ground.

### 2.2 Extraction

The cannabis stems were extracted with solvent by maceration process: hexane, 95%ethanol, 80%Methanol and distilled water at a ratio of 1 to 3 (w/v). the extraction time as 7 days and filled with Whatman No1. The eluate was evaporated to remove solvents with Rotary evaporator (Temperature: 60°C, Pressure: 110 mm.Hg. and Rotation rate: 80 rpm).

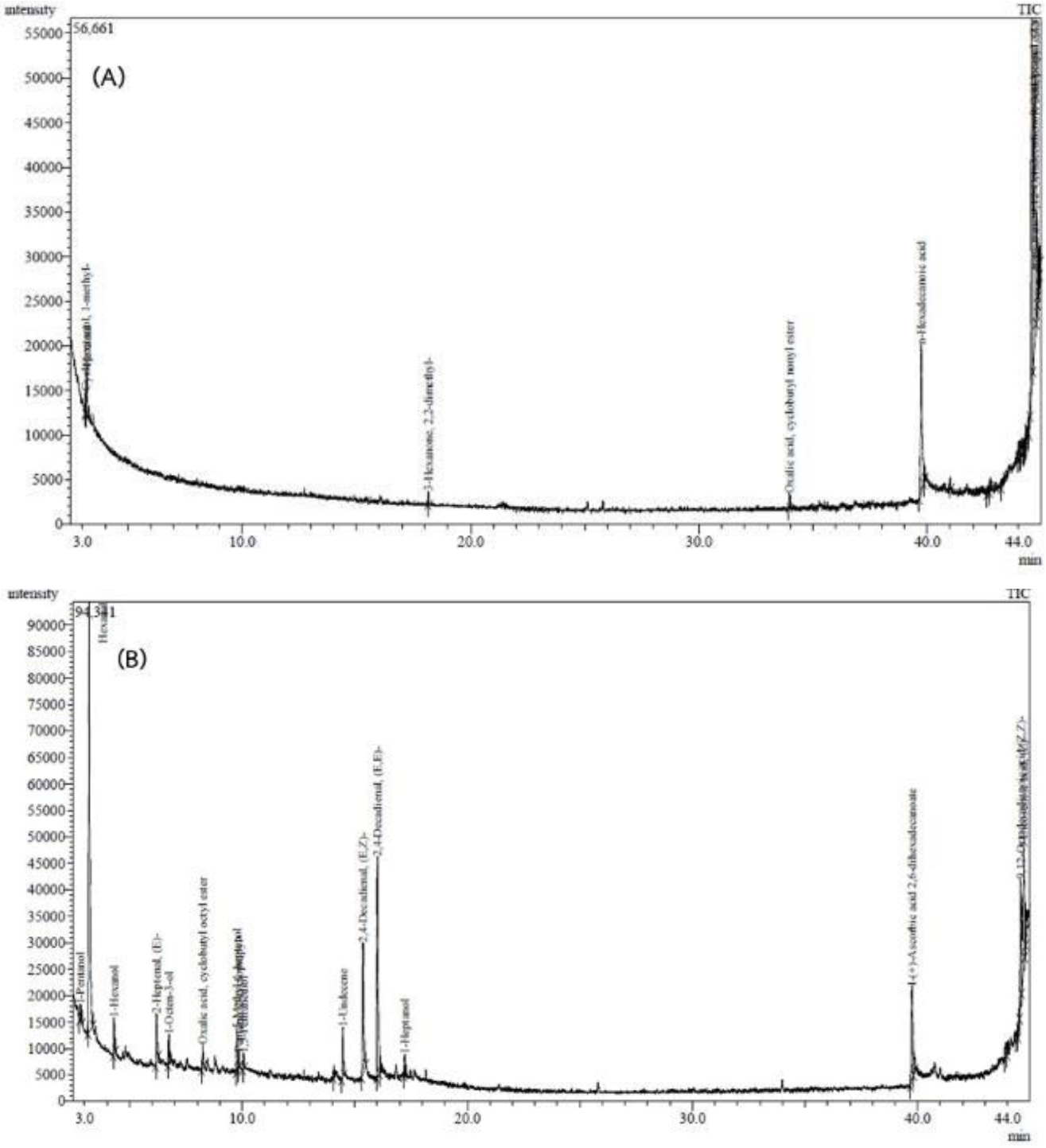
**2.3 GC-MS analyses of Chemical Constituents**

The crude extracts were dissolved with methyl alcohol at a concentration of 0.2 mg/mL. The sample substance was introduced into the analytical process with GCMS-QP2020 Ultra (Shimadzu) in the study, sh-Rtx-5MS capillary column was used for analysis (length 30 meters x diameter 0.32 micrometers x film thickness of 0.25 micrometers; The temperature does not exceed 330 degrees Celsius and uses mass spectrometry (MS model QP2020 Ultra: Shimadzu) under carrier gas, helium gas, and the analytical temperature is starting from 50°C, then added to the 140°C with the ramp rate of 5°C/min and maintained at 140°C for 5 minutes and added to the 200°C with the ramp rate of 3°C/min and added to the 260°C with the ramp rate of 15°C/min. GC-Ms technical analysis modified method from Muhammad Tayyab (Andre et al., 2016)

The sample injection was 1 μL, with spilt ratio of 50, the collecting analytical signals in the range of 35 - 400 amu with ion-inducing energy 70eV. The total time for analysis was 45 minutes. The chemical composition of the crude extract was considered the percentage of the by using the area of GC-MS chromatogram (Peak area) and identified the chemical compound based on retention time and Mass spectrum, with the Library NIST17 database.

**3. Results and Discussion**

Chemical composition analysis of extracts with GC-MS techniques can be used to predict the chemical essence in the sample substance quite accurately based on the comparison. The fingerprint of the mass number of the sample substance was used as the tool's existing database, which can be analyzed the properties of quantitative and qualitative. The chemical composition from the crudes of cannabis stems were extracted by hexane solvent, 95% ethanol, 80% Methanol and distilled water. The GC-MS techniques were used for characterization of the chemical compounds. The performing results were analyzed by determining the composition compared with the database of the Library NIST17. (Figure. 1 A-D)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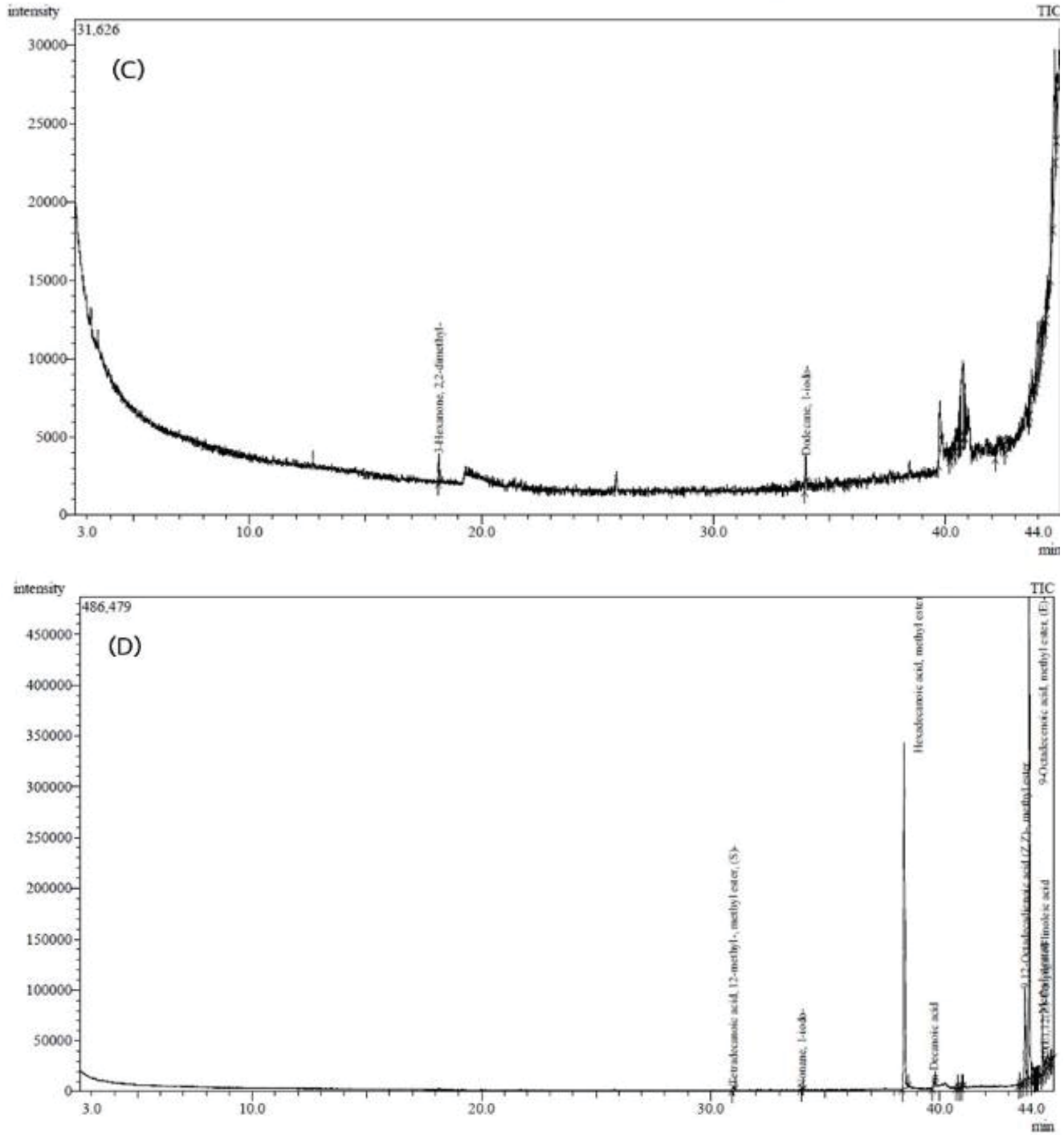


Figure. 1 GC-MS chromatogram of (A) the Hexane extract, (B) the 95% Ethanol extract, (C) the 80% Methanol extract and (D) the distilled water extract of cannabis stems.

Table 1 The phytochemical compounds detected in (A) the Hexane extract, (B) the 95% Ethanol extract, (C) the 80% Methanol extract and (D) the distilled water extract of cannabis stems by GC-MS.

| Solvents | Compounds | R. time(min) | % Area | Pharmacological action |
|---|---|---|---|---|
| Hexane | Cyclopentanol, 1-methyl- | 3.13 | 0.62 | essential oils antibacterial (Muthulakshmi. et al., 2012) |
| | Hexanal | 3.19 | 3.62 | Volatile compounds (Andre et al., 2016) antimicrobial activity (Awizen, 2014) |
| | 3-Hexanone, 2,2-dimethyl- | 18.16 | 0.56 | Antimicrobial Activity (Osama, 2017) |
| | Oxalic acid, cyclobutyl nonyl ester | 3.97 | 0.52 | retarded cell growth and metastasis of cancer cells (Wintola & Afolayan, 2017) |
| | n-Hexadecanoic acid | 9.74 | 14.66 | essential oil, antimicrobial agents [6], Anti-inflammatory (Devi & Singh, 2021) |
| | trans,trans-9,12-Octadecadienoic acid, propyl ester | 4.58 | 31.4 | medicinal uses (Dandashire. et al., 2019) |
| 95% Ethanol | 9-Octadecenal, (Z)- | 4.69 | 30.56 | Phytopharmaceutical (Chowdhury. et al., 2017) |
| | 1-Pentanol | | 1.10 | Nr |
| | Hexanal | | 25.09 | Volatile compounds (Andre et al., 2016) |
| | 1-Hexanol | | 1.51 | Volatile compounds (Wintola & Afolayan, 2017) |
| | 2-Heptenal, (E)- | | 3.13 | essential oil, antimicrobial agents (Bryant & McClung. et al., 2011) |
| | 1-Octen-3-ol | | 1.13 | Nr |
| | )xalic acid, cyclobutyl octyl ester | | 1.24 | retarded cell growth and metastasis of cancer cells (Wintola & Afolayan, 2017) |
| 80% Methanol | 3-Hexanone, 2,2-dimethyl- | 18.17 | 1.44 | Antimicrobial Activity (Dandashire. et al., 2019) |
| | Dodecane, 1-iodo- | 33.98 | 2.86 | Nr |
| Distilled water | Tetradecanoic acid, 12-methyl-, methyl ester, (S)- | 31.00 | 0.39 | antioxidant, anti-cancer activity, and hypo-cholesterolemic activities (S TL. M. et al., 2021) |
| | Nonane, 1-iodo- | 33.98 | 0.15 | Nr |
| | Hexadecanoic acid, methyl ester | 38.46 | 39.15 | Antimicrobial Activity (Devi & Singh, 2021) Anti-inflammatory (Mohiuddin. et al., 2018) |
| | n-Decanoic acid | 39.75 | 0.66 | Antibacterial, anticancer, Antioxidant (Kishimoto. et al., 2007) |

Table 1 The phytochemical compounds detected in (A) the Hexane extract, (B) the 95% Ethanol extract, (C) the 80% Methanol extract and (D) the distilled water extract of cannabis stems by GC-MS. (Continous)

| Solvents | Compounds | R. time(min) | % Area | Pharmacological action |
|---|---|---|---|---|
| Distilled water | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 43.73 | 8.20 | Antioxidant activity (Thirumalai. et al., 2021) |
| | 9-Octadecenoic acid, methyl ester, (E)- | 43.91 | 39.04 | Antioxidant activity (Thirumalai. et al., 2021) |
| | Methyl stearate | 44.49 | 4.17 | antioxidant activity (Thirumalai. et al., 2021) |
| | 10(E),12(Z)-Conjugated linoleic acid | 44.58 | 1.06 | medicinal uses (Mishra & Patnaik, 2020) |

Note: not reported (Nr)

The composition of essential compounds from the analysis results are given in Table1. The consistency of extracts derived from different solvents can be found: Hexanal are found by Hexane and 95% Ethanol. 9,12-Octadecadienoic acid (Z,Z)- derived from crude extracts of 95% ethanol and distilled water solvents, respectively.

The GC-MS results were compared with previous studies. The chemical constituents were composed of important substances to considering the phytochemicals (Muthulakshmi. et al., 2012). as the retarded cell growth and metastasis of cancer cells (Wintola & Afolayan, 2017) antimicrobial agents (Bryant & McClung. et al., 2011), anti-inflammatory (Dandashire. et al., 2019)and antimicrobial, antioxidants (Dandashire. et al., 2019) (Devi & Singh, 2021) (Mishra & Patnaik, 2020) (Mohiuddin. et al., 2018) (Asghar. et al., 2013) (Thirumalai. et al., 2021) (S TL. M. et al., 2021) (Kishimoto. et al., 2007), respectively. The volatile compounds (Bryant & McClung. et al., 2011) and phytochemicals were found, that applied in medicinal used (Dandashire. et al., 2019). The crude extract results applied for the selection of the suitable solvent for extraction and the active substances in order to utilization based on the pharmacological properties

## 4. Conclusions

The results of GC-MS revealed the phytochemicals of cannabis stems in the group of the retarded cell growth and metastasis of cancer cells, antioxidant, antimicrobial and anti-inflammatory, respectively. The volatile compounds and medically used compounds, that were determined. The results were the database for the application of cannabis stems, the selection of the suitable solvents and the chemical constituent actions.

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