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Maceration Mediated Extraction of Marijuana Leaves Oil and Separation of Δ9-Tetrahydrocannabivarin (THCV) by Thin Layer Chromatography

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A B S T R A C T

Cannabidiol (CBD), non-psychoactive cannabinoid, was extracted from Cannabis sativa leaves by using solvent extraction method followed by reflux condenser procedure. Physical and chemical properties of Cannabidiol (CBD) were determined. Various parameters and contents of extracted oil were analyzed. Two different samples were prepared by applying different conditions. Sample "A" was prepared by applying reflux condenser and sample "B" was prepared by soaking eaves in ethanol (solvent) for one week. Various parameters and contents of extracted oil were analyzed. TLC, UV, GC-MS, IR and HPLC techniques were applied for characterization of both samples and oil. Some tests were also performed for the detection of proteins, carbohydrates, Steroids, tannins, gums and mucilage. Acid value, saponification number and specific gravity were also calculation by applying titration method

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INTRODUCTION

Cannabis sativa or marijuana is a plant that is very popular around the world. Cannabis sativa is a tall plant with thin leaves that can reach a height of 518 feet and is frequently branchless (Li et al., 2020). *Cannabis sativa* is a prolific generator of terpenes and terpenoids (Nelson et.al, 2020). It has a remarkable capacity for the production of numerous secondary metabolites from various chemical classes, despite its complex chemical makeup. Given their pharmacological importance, cannabinoids are among them and have received the most research (Brighenti et.al, 2021). The existence of secondary substances referred to as "cannabinoids," or more perfectly "phytocannabinoids," is one characteristics shared by all marijuana plants. The majority of the phytocannabinoids, which number over 100, are created in the trichomes that develop on female Cannabis inflorescences. But the composition and abundance of certain phytocannabinoids varies greatly between Cannabis varieties (Schilling, Mazler, and Mccabe, 2020). By National Cancer Institute cannabinoids are defined as "a sort of molecule in marijuana that induces medicinal effects throughout the body, including the brain and immunological system". There are at least 100 more minor cannabinoids in marijuana, including delta-9-tetrahydrocannabinol (delta-9-THC), cannabidiol (CBD) and more (Odell, Tuell, Shah and Stone, 2022).

Cannabidiol (CBD), cannabichromene (CBC), and cannabigrol (CBG) are non-psychoactive cannabinoids with a range of medicinal effects, together with additional non-cannabinoid components from a number of natural sources (ElSohly, Radwan, Gul, Chandra and Galal, 2017). CBD has a favorable effectiveness and safety and is generally accepted in people. Preclinical research suggests that CBD may be therapeutically effective for curing a wide range of illnesses.like multiple sclerosis symptoms, anxiety, depression, cancer, cardiovascular disease, neurodegeneration, psychotic symptoms, seizure disorders, psychotic symptoms, anxiety, depression, inflammation, and chronic pain (Corroon and Phillips, 2018).

LITERATURE REVIEW

The terpenophenol structure of Cannabidiol (CBD) was determined in 1963, having molecular weight of 314.464 g/mol with 21 carbon atoms and molecular formula $C_{21}H_{30}O_2$ (Atalay, Karpowicz & Skrzydlewska, 2020). The -OH groups of the phenol ring are thought to be mainly responsible for the opposite of oxidative activity of CBD (Borges et al., 2013). Nuclear and Ionotropic receptors, as well as cannabinoid (CB1 and CB2) receptors, can all be activated, antagonistic, or inhibited by CBD, depending on the dose (Ghovanloo, Shuart, Mezeyova, Dean, Ruben and Goodchild, 2018).

In order to obtain FDA clearance for CBD separated from marijuana for the treatment of two paediatric epilepsy disorders, a New Drug Application was filed to the agency in October 2017. Epidiolex (cannabidiol) became the first cannabis chemical produced from a plant to get FDA drug approval in June 2018 after receiving approval (Keating, 2017). The legal situation of CBD is complicated and evolving globally. CBD is permitted in many nations as a component of the prescription drug Sativex (nabiximols) (Abuhasira, Shbiro and Landschaft 2018).

METHODOLOGY

The leaves of plant cannabis sativa were taken from the jungle of Changa Manga. The leaves of plant *Cannabis sativa* were collected from the branches carefully. No seeds and other branch parts were mixed with leaves. The leaves were washed, before to use, dried and crushed. After drying, the leaves were crushed into small pieces. The small pieces of leaves were divided into two parts. One of the parts was taken in round bottom flask and preceded further through reflux process by condenser. The round bottom flask was filled with small pieces of leaves and methanol and covered with aluminum foil and thermometer adjusted in it. The flask was put on the heating mantle and rubber pipes for water in and water out were attached. The heating mantle was turned on. The mixture of leaves and solvent was started heating and boiling. The solvent start evaporated and collected in another flask. The process is repeated again and again up to 3 hours. After 3 hours, the apparatus was removed and the oil kept for cooling. After filtration this oil was marked as sample A.

Other part was taken in seven conical flasks filled with solvent methanol and kept for one week. The filtrate from the seven conical flasks was separated from the leaves residue and collected in a beaker. Then beaker was covered with aluminum foil with adjusted thermometer in it. The beaker was put on the hot plate and temperature was strictly measured. The beaker was heated till third half of solvent was evaporated and only oil was remained. Then oil was stored in air tight container. This oil was marked as sample B.

The evaluation and characterization of both samples was done. The evaluation includes the determination of saponification number, acid value, specific gravity and refractive index. The saponification number and acid value was determined by titration procedure, specific gravity was determined by pcynometer and refractive index was calculated with the help of refractometer. IR, UV, HPLC and TLC were performed for the characterization of the samples. Different physical tests were performed for the determination of alkaloids, proteins, Saponins, reducing sugar, gums and mucilage. The thin layer chromatographic technique was employed to determined the % of extracted CBD from oil.

RESEARCH RESULT

It was observed that the oil produced from soaked leaves has excellent and good qualities. The values obtained for density, viscosity, refractive index, saponification, acid, and iodine of sample "A" were 12.411, 1.622, 140.35 and 39.83 respectively and for sample "B" was 6.511, 1.680, 138.45 and 19.07. These values were approximately in agreement with previous research.

The physical test performed for proteins, alkaloids and reducing shown positive results while test for Saponins and tannins showed negative result. Same results were shown by Audu, Ofojekwu, Ujah and Ajima (2014).

The IR- chromatogram showed three main peaks which showed three main functional groups like alcohol, alkane and alkene as given in CBD structural formula. Same results of IR were shown by Haldhar, Prasad, Mandal, Benhiba, Bahadur and Dagdag (2021). The maximum absorption at 275 nm by CBD shown by UV-chromatogram matched by Ryu et.al, (2021) and by HPLC chromatogram CBD showed peak at retention time of 3.692.

Cannabidiol (CBD) yield was extracted by solvent extraction method and determined by thin layer chromatographic technique. Maximum yield is obtained by the solvent extraction method. Solvent extraction equipment provides a maximum yield of about 36.77%. Solvent aqueous method don't not generate significant yield.

CONCLUSIONS AND RECOMMENDATIONS

Solvent extraction method was utilized to extract the Cannabidiol (CBD) from the leaves of *Cannabis sativa*. Oil content was %. Physiochemical properties for Cannabidiol (CBD) were studied. Before and after modification the extracted Cannabidiol (CBD) oil was analyzed for determination of PH, acid value, Sponification value, refrective index, specific gravity, viscosity and iodine value etc. Their respective values of aformentioned parmeters for oil obtaind from soaked leaves and condensed leaves were determined. 7.5 (PH), Oxadative stability depends upon the unsaturation contents of oil. Modified oil can be used for various medicinal purposes.

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