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Review Article

Biological Activity of *Cannabis sativa* L.

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Abstract

Hemp (*Cannabis sativa*) is a valuable plant that can be used in its entirety, has economic value in many different areas, and is superior in almost all areas of use. It is a dioecious annual plant that is becoming more and more valuable for the pharmaceutical industry. Due to its fibers, seeds and especially the cannabinoids it contains, the number and quality of academic studies on this subject have been rapidly increasing recently. Phytochemical content is one of the most important factors in hemp production. The phytochemicals of *Cannabis sativa* are quite complex and hundreds of compounds from different chemical classes have been identified. In hemp, amino acids, fatty acids and steroids represent the primary metabolism, while cannabinoids, flavonoids, stilbenoids, terpenoids, lignans and alkaloids represent secondary metabolites. *Cannabis sativa*, which has the potential to contribute to the country's economy, has features that encourage use in both industry and medical fields, causing the plant to be considered as the plant of the future.

Keywords: Hemp, Antioxidant activity, THC, Essential oil, Antimicrobial activity

1. INTRODUCTION

Nowadays, interest in plant-derived antioxidants is increasing. Due to its natural origins, it is considered non-toxic, safe for humans and the environment. Therefore, the application of natural antioxidants, such as dietary antioxidants, nutritional supplements or functional nutritional ingredients, is viewed as more environmentally friendly and more preferred by consumers [1]. As an important plant source, studies on the antioxidant activity of the hemp plant are limited [2]. Hemp is also used for medical and nutritional purposes. Hemp has great economic importance as it is used in the production of textiles, paper, and household goods. Hemp is generally researched in two classes: industrial and medical hemp [3]. Hemp (*Cannabis sativa* L.), belonging to the Cannabinaceae family, is known as an annual and generally dioecious plant with palmate leaves and is cultivated in Türkiye [4]. Hemp cultivation is done under control due to the psycho-active substances obtained from female hemp. All types of hemp contain components containing narcotic properties, especially *Cannabis indica* species contain the most narcotic components and are grown mostly to obtain narcotic substances. Hemp, whose vegetative growth rate increases with the increase in day length, transitions to the flowering phase with the onset of short days. Although the growing period of the plant is 4-6 months, the variability of factors such as temperature, hormonal control, photoperiod, and stress can increase or shorten the growing period of the plant. The site of synthesis and storage of cannabinoids is the glandular trichomes (galdular hairs). The most important reason why the amount of cannabinoids in female plants is generally higher than in males is that they have more glandular trichomes [5]. Glandular hairs, which have the functionality to increase the adaptation of plants to their environmental conditions, to be a physical barrier against external factors (pathogens, animals, etc.), as well as to protect against extreme temperatures and ultraviolet radiation, can be seen in various structures and shapes originating from the epidermal cells of the plant. In hemp, glandular hairs are the primary structures for synthesizing and storing cannabinoids [6]. The female inflorescences and leaves of hemp are covered with glandular trichomes, which are the highest concentrations of phyto-cannabinoids and their ability to synthesize secondary metabolites [7]. Hemp contains many phytochemicals (amino acids, fatty acids and steroids, phytocannabinoids, terpenes and phenolic compounds) belonging to different chemical classes. Their concentrations vary depending on both plant characteristics (tissue type, age, etc.) and environmental factors (temperature, humidity, light, etc.) [4]. Fiber, essential oils and seeds are obtained from different parts of the plant [8]. In recent years, interest in the use of industrial hemp seeds for oil and flour production has increased and its cultivation has become widespread all over the world. Thanks to its root systems, it is very resistant to drought and also to pests. It requires less water than other fiber plants, grows very

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quickly and can be widely cultivated in many geographies [9]. Lignocellulosic fibers are among the highest quality fibers and also have antibacterial properties. For this reason, it is very suitable for the production of antibacterial textile products and surgical materials [8].

2. CHEMICAL CONTENT OF HEMP

Hemp, whose phytochemical content is very complex, contains many compounds belonging to different chemical classes. Most of the chemical compounds contained in hemp are produced through secondary metabolism. Secondary metabolites are obtained by synthesizing primary metabolism products. The versatile roles of secondary metabolites such as natural selection, ecology, and their relationships with plant-animal microorganisms are also very important. *C.sativa* has a variety of chemical compounds, including such as cannabinoids (cannabigerol, cannabichromene, cannabidiol, cannabisykosol, cannabielsoin, cannabinol, cannabinodial, cannabitriol), enzymes, amino acids, proteins, glycoproteins, sugars, hydrocarbons, nitrogenous components, simple esters, fatty acids, lactones, steroids, terpenes, phenols, flavonoids, vitamins, pigments and elements. While amino acids, fatty acids and steroids found in the plant belong to primary metabolism; Phytocannabinoids, terpenes and phenolic compounds are known as metabolites of secondary metabolism. The main cannabinoids of the cannabis species are THC (Δ9-tetrahydrocannabinol), CBD (Cannabidiol), CBDA (Cannabidiolic acid), CBG (Cannabigerol), CBGA (Cannabigerolic acid), CBGV (Kannabigevarin) and CBGVA (Cannabigevaric acid) [10]. Receptors that do not activate most cannabis compounds are also often called cannabinoids. However, (Δ9-tetrahydrocannabinol is the main compound that is the psychoactive substance. Particularly two of the phytochemical substances contained in *C. sativa* L., THC and CBD, find use in terms of their pharmacological properties. Among the cannabinoids, the compound with the highest antioxidant activity and other biological activities is cannabidiol (CBD). Formation of CBD; The biosynthesis of cannabinoids occurs by the degradation of THC and its storage for a long time. [11]. THC and CBD are responsible for the therapeutic effects of hemp are its two main active ingredients. While THC is psychoactive, CBD is not psychoactive. However, this is not the case for all cannabis varieties. However, this is not the case for all cannabis varieties. While the fiber-rich hemp variety usually contains low amounts of psychoactive THC, the medically used hemp variety has a much higher THC content [4]. It has attracted the attention of pharmaceutical companies as a result of studies showing that CBD does not have psychoactive properties and, on the contrary, can be effective in the treatment process for many diseases such as cancer, epilepsy and Parkinson's [12]. It also contains more than 545 known compounds that give cannabis its distinct medicinal properties [13]. In the cannabis plant, cannabinoids are synthesized and stored in glandular hairs.

Cannabinoids synthesized in the glandular hairs are stored in the secretory space between the cuticle and the disc cells that form the head of the glandular hairs [14]. Cannabinoid content varies in terms of quantity and quality. The amount of cannabinoids is affected by environmental factors. Cannabinoid quality is largely genetic [15]. *C.sativa* has five different chemotype groups based on their cannabinoid profiles. Chemotype-I contains large amounts of Δ^9-tetrahydrocannabinol (Δ^9 THC), while chemotypes-III and IV are fiber-producing plants with high amounts of non-psychoactive cannabinoids. While chemotype-II is found among plants with high drug and fiber content, chemotype-V plants produce fiber but do not contain cannabinoids [16-17]. Flavonoids are products found everywhere in the plant body and have many functions in plant biochemistry, physiology and ecology [18]. Terpenes and terpenoids are important components of medicinal aromatic plants, including cannabis, that have pharmacological effects, including resins and essential oils. Terpenes, unlike terpenoids, are basic hydrocarbons. It contains functional groups of a wide variety of chemical elements. However, terpenes and terpenoids are often used interchangeably. Terpenes constitute the largest phytochemical group. Terpenes have been reported to be isolated from the essential oil obtained from flowers, roots, and leaves [19]. Terpenes are widely used in industry, perfumery, as food additives and in traditional medicines. Cannabis plants typically consist of terpenes up to 3-5% of the dry mass of the female inflorescence. Hemp terpenes are typically simple mono- and sesquiterpenes derived from two and three isoprene units, respectively. Terpenoids or isoprenoids are another important group of major plant metabolites. Isoprenoids function as plant hormones (abscisic acid, cytokinin’s and gibberellic acid) in primary metabolism, are an important component of the cell membrane (sterols), carry oxygen to cells and play an effective role in photosynthesis (Table 1). Secondary metabolites they take part in transmission and plant defence mechanisms [20]. Alkaloids are another important secondary metabolite in plants. However, alkaloids are a very large and heterogeneous group of compounds that can be obtained not only from plants but also from microorganisms, insects, and animals. Although alkaloids are basic, they are generally nitrogen compounds with biological activity at low doses and are obtained from amino acids. 10 alkaloids have been identified in cannabis. These alkaloids have been isolated from the stem, leaves, roots, pollen and seeds of cannabis [7,11]. In plants, alkaloids often serve as a defence against predators due to their toxicity, bitter taste, and effects on the central nervous system, resulting in increased species survival [20]. Although the effects of alkaloids on human physiology are diverse, some alkaloids affect the nervous system and sometimes cause hallucinations, while others affect the muscles. Due to these negative properties, alkaloids can even cause death. On the other hand, they constitute one of the raw materials of important medicines [22].

Table 1. Pharmacological properties of *Cannabis sativa* described in the literature.

S.N.	Part used	Extraction method	Pharmacological properties	Compounds	References
1	Stem, leaves, seeds, flowers	Hydro-distillation and solvent extraction	Antiplasmodial activity, anti-inflammatory	Beta-myrcene (15%)	[23], [24], [25]
2	Steam	Hydro-distillation	Anti-tumour, Antioxidant, and antimicrobial activities	α-pinene (16.4–18.2%)	[26]
3	Steam	Hydro-distillation	Anti-inflammatory	α-humulene (6.9–8.3%)	[26], [27]
4	Whole plant	Ethanol extracts	Analgesic and anti-inflammatory	Linalool (10.06%)	[28]
5	Flowers, bracts, and peduncle	Steam distillation	Analgesic and anticancer activities	β-caryophyllene oxide (19.71%)	[29]
6	Steam	Hydro-distillation	Anticancer, anticoagulation, antimalarial, antimicrobial, and antioxidant effect	β-pinene (5.2%)	[26], [27]

3. BIOLOGICAL ACTIVITIES OF HEMP

3.1 Antimicrobial Activity

The use of plants in healing diseases dates back almost to the era of humanity. Throughout human history, different forms of plants have been turned into poultices and applied to open wounds, boiled, and consumed as food, or consumed directly as food. When we look at the world in general, approximately half of the deaths in tropical countries are caused by infection. Every year, 300,000 children in Africa die from infections caused by microorganisms related to *E. coli*, *Shigella* and *Salmonella* species. Perhaps this situation is not surprising considering the socio-economic status of the countries, but infection-related diseases and deaths are increasing day by day in developed countries [30]. Therefore, it has become necessary to develop new strategies in the prevention and treatment of infectious diseases. For this reason, it is quite natural that pharmacologists and especially microbiologists turn to plants in search of antimicrobial agents. Over time, microorganisms acquire resistance to drugs and transmit them to new members [31]. In this respect, the antimicrobial effect of the hemp plant has begun to be investigated in recent years. Hemp seed oil contains moderate to high amounts of tocopherols and tocotrienols, phytosterols, phospholipids, carotenes, and minerals [32]. These properties of hemp seed oil allow for numerous potential applications as a functional ingredient in foods and in the treatment of many different health problems [33].

A recent study suggested that hemp seed oil can be used in antimicrobial research against Gram-positive bacteria [34]. *C. sativa* was extensively studied by many researchers [35–47] and reported the antimicrobial activities. According to the in vitro study, the potential antimicrobial effect of *C. sativa* leaf extracts containing ethanol, chloroform, acetone, and water is effective against *P. aeruginosa*, *E. coli*, *S. aureus*, and *A. niger* tested against those positive results delayed the growth of the tested microbes. According to the results, acetone and ethanol extracts showed maximum inhibition zone against most of the tested strains. Cannabis chloroform extract exhibited lower activity against the microbial strain [42]. In another study, ethanol, methanol and chloroform extracts were prepared from *C. sativa* leaves. The highest inhibition zone was detected in the chloroform extract of *Cannabis sativa* against *S. typhi*, followed by the ethanol extract against *S. aureus* [43].

3.2 Antioxidant Activity

Nowadays, due to increasing concerns about the reliability of synthetic antioxidants, there has been an increasing interest of the health and food industry in obtaining natural antioxidants from medicinal and aromatic plants. Antioxidants are molecules that enable free radicals to become stable by providing them with suitable electrons. In recent years, interest in natural antioxidant source plants has increased greatly, and therefore the number of studies investigating the use of medicinal and aromatic plants as natural antioxidant sources continues to increase day by day. Studies on this subject have shown that medicinal and aromatic plants contain many phytochemical compounds with high levels of antioxidant activity [48]. Until recent years, studies on phenol and polyphenol components in hemp seeds and by-products were scarce [49]. Even today, the polyphenol content and functions in hemp seeds as well as other parts of the hemp plant are not fully understood. It has been reported in many studies that seeds and other parts of the plant have high antioxidant potential [49–53].

According to studies, the antioxidant activity of extracts obtained from the hemp plant has been evaluated by many researchers and it has been determined that they show strong antioxidant activity [54–59] (Table 2). The main polyphenols found in the seed, N-trans caffeoyltiramine, phenylpropanoids amides, vanillic acid, gallic acid, protocatechuic acid, ferulic acid, sinapic acid, and cannabisin were characterized. It was concluded that *C. sativa* would be a suitable source of polyphenols for nutraceutical or supplement purposes [60,61]. In recent years, phenols and polyphenols, have received much attention due to their potential use as prophylactic and therapeutic agents in many diseases. These compounds to slow down and actively neutralize oxidative stress has led researchers to work toward disease prevention [60].

The best-characterized polyphenolic component in cannabis fruits is phenylpropanoid amides and their derivatives, also called phenylamides or hydroxycinnamic acid amides [61]. These compounds are defined as bioactive because they exhibit multiple pharmacological activities [62]. According to the data in the literature, it has been emphasized that the presence of large amounts of antioxidant polyphenols in hemp seed oil is necessary not only for the protection of the consumers, but also for the preservation of the oil [63]. Hemp seeds are a candidate to be an important source in terms of macro nutrients content and quality. It is considered an important industrial product whose production has been permitted in some countries, especially with the development of varieties containing low physicoactive substances. It is considered a promising product in many respects due to the antimicrobial, antihypertensive, cancer and heart disease-preventing and shelf-life-extending effects of the phytochemicals in the seeds [64]. In addition to their nutritional value, hemp seeds are also rich in natural antioxidants. The bioactive compounds of hemp seed oily fraction are tocopherols [8]. Other bioactive compounds in hemp seed oil are represented by carotenoids, can act as antioxidants, and reduce the risk of degenerative diseases [9]. It shows that the antioxidative effects of hemp seed dietary supplement can improve the redox state under certain conditions [65]. According to another study, after the intervention of different concentrations of hemp oil in the diets of Teddy dogs, total protein, albumin and globulin, immunoglobulin E and γ interferon (IFN-γ) significantly increased in the biochemical parameters of dogs. In addition, hemp oil improved superoxide dismutase and reduced malondialdehyde [66]. In a study analysing the effects of hemp seed oil on oxidative stress markers and the life cycle of *D. melanogaster* (vinegar fly/fruit fly), under oxidative stress conditions, hemp seed oil showed beneficial effects on oxidative stress markers [65].

Table 2. Antioxidant activity of *Cannabis sativa* [72]

S.N.	Part Used	Extract Used	Method	Reference
1	Leaves, Stem, and Inflorescence	chloroform, n-hexane, ethyl acetate	ABTS, DPPH, TAEC	[54]
2	Leaves and stems	ethanol and methanol	DPPH	[41]
3	Whole plant	aqueous	DPPH	[67]
4	Leaves and roots	Ethyl acetate,Ethanol, acetone, chloroform, distilledwater and methanol	DPPH	[56]
5	Seeds	methanol	DPPH	[57]
6	Leaves	Aqueous	ABTS, DPPH	[59]
7	Seeds	Hexane	DPPH	[68]

8	Seeds	MethanoL	Total phenolic content, ABTS	[69]
9	Seeds	Methanol	Total phenolic content, DPPH, total flavonoid content, ABTS	[70]

DPPH: 2,2-difenil-1-pikrilhidrazil; TAEC: Trolox equivalent antioxidant capacity; ABTS: 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid.

According to the study, the results obtained from antioxidant tests showed a moderate effect on C. sativa essential oil (IC_{50} = 1.6 mg/m L for the DPPH test and IC_{50} = 0.9 mg/m L for the ferric reducing power test). Total phenolic content of hemp seed extract was found to be 2.21 mg GAE/g FW [72]. In the study where the chemical components of the clusters of eight different fiber-type hemp varieties grown in Switzerland were determined for different planting periods throughout the 2019 cultivation year, the main findings were that TPC, as well as individual flavonoids and terpenes, were mainly affected by the harvest period and the phenological stage of the plant [73]. Due to the presence and effects of bioactive components in hemp, it has been used in the treatment of many diseases in recent years. These diseases include cancer, epilepsy, Alzheimer's, schizophrenia, diabetes, sleep problems, chronic pain, chemotherapy-related nausea and vomiting, anorexia, anxiety, and multiple sclerosis.

4. CONCLUSION

Hemp is an important plant known to humanity for centuries. With the studies carried out in recent years, its usage areas are also increasing. Its use in industry is particularly noteworthy. In terms of biological activity, it is seen that it is used in the treatment methods of many diseases. It is observed that there is a need to determine the broader biological activities of cannabis both in vivo and in vitro. Since hemp is also used as a drug, its production and consumption should be done in a controlled manner. It should be prevented from being used for bad purposes, but its beneficial aspects should also be utilized. Therefore, its use should be increased in industry, pharmaceutical, cosmetics, textile, furniture and construction sectors. It is seen that hemp extracts are used in the production of many biological drugs. For this purpose, both the existing drug potential and the potential for use of new drugs are considered very important. The concentrations of compounds present depend on plant tissue, age, genotype, growing conditions (nutrient, moisture, and light amount), harvest time and storage conditions. These compounds are very important pharmacologically, but they have not been adequately researched scientifically.

REFERENCES

[1] M. Kalinowska, A. Płońska, M. Trusiak, E. Gołębiewska, and A. Gorlewska-Pietluszenko, "Comparing the Extraction Methods, Chemical Composition, Phenolic Contents and Antioxidant Activity of Edible Oils from Cannabis sativa and Silybum marianu Seeds," Sci. Rep., vol. 12, no. 20609, pp. 1–16, Nov. 2022, doi: 10.1038/s41598-022-25030-7.

[2] K. Ning, C. Hou, X. Wei, Y. Zhou, S. Zhang, Y. Chen, and S. Chen, "Metabolomics Analysis Revealed the Characteristic Metabolites of Hemp Seeds Varieties and Metabolites Responsible for Antioxidant Properties," Front. Plant Sci., vol. 13, no. 904163, pp. 1–11, Jun. 2022, doi: 10.3389/fpls.2022.904163.

[3] S. A. El-Sohaimy, N. V. Androsova, A. D. Toshev, and H. A. El Enshasy, "Nutritional Quality, Chemical, and Functional Characteristics of Hemp (*Cannabis sativa* ssp. sativa) Protein Isolate," Plants, vol. 11, no. 21, pp. 1–13, Oct. 2022, doi: 10.3390/plants11212825.

[4] A. Gökgöz and E. Y. Can, "Medikal ve Endüstriyel Açıdan Kannabinoidlerin Önemi ve Türkiye Ekonomisine Katkı Potansiyeli," Med. J. West Black Sea, vol. 5, no. 3, pp. 315–323, Apr. 2021, doi: 10.29058/mjwbs.928899.

[5] O.V. Razumova, O.S. Alexandrov, M. G. Divashuk, T. I. Sukhorada, and G. I. Karlov, "Molecular Cytogenetic Analysis of Monoecious Hemp (*Cannabis sativa* L.) Cultivars Reveals its Karyotype Variations and Sex Chromosomes Constitution," Protoplasma, vol. 253, no. 3, pp. 895–901, May. 2016, doi: 10.1007/s00709-015-0851-0.

[6] E. Werker, "Trichome diversity and development," vol. 31, pp. 1–35, Jan. 2000.

[7] S.A. Bonini, M. Premoli, S. Tambaro, A. Kumar, G. Maccarinelli, M. Memo, and A. Mastinu, "*Cannabis sativa*: A comprehensive Ethnopharmacological Review of a Medicinal Plant with a Long History," J. Ethnopharmacol. vol. 227, pp. 300–315, Dec. 2018, doi: 10.1016/j.jep.2018.09.004.

[8] M. Irakli, E. Tsaliki, A. Kalivas, F. Kleisiaris, E. Sarrou, and C. M. Cook, "Effect of Genotype and Growing Year on the Nutritional, Phytochemical, and Antioxidant Properties of Industrial Hemp (*Cannabis sativa* L.) Seeds," Antioxidants, vol. 8, no. 491, Oct. 2019, doi: 10.3390/antiox8100491.

[9] F. Blasi, C. Tringaniello, G. Verducci, and L. Cossignani, "Bioactive Minor Components of Italian and Extra-European Hemp Seed Oils," Lwt, vol. 158, no. 113167, Jan. 2022, doi: 10.1016/j.lwt.2022.113167.

[10] C. M. Andre, J. F. Hausman, and G. Guerriero, "*Cannabis sativa*: The Plant of the Thousand and One Molecules," Front. Plant Sci., vol. 7, no. 174167, Feb. 2016, doi: 10.3389/fpls.2016.00019.

[11] C. E. Turner, M. A. Elsohly, and E. G. Boeren, "Constituents of *Cannabis sativa* L. XVII. A Review of the Natural Constituents," J. Nat. Prod., vol. 43, no. 2, pp. 169–234, Mar. 1980, doi: 10.1021/np50008a001.

[12] E.M.J. Salentijn, Q. Zhang, S. Amaducci, M. Yang, and L. M. Trindade, "New Developments in Fiber Hemp (*Cannabis sativa* L.) Breeding," Ind. Crop. Product, vol. 68, pp. 32–41, Jun. 2015, doi: 10.1016/j.indcrop.2014.08.011.

[13] M. A. Elsohly, M. M. Radwan, W. Gul, S. Chandra, and A. Galal, "Phytochemistry of *Cannabis sativa* L. In: Kinghorn A., Falk H., Gibbons S., Kobayashi J. (eds.), Phytocannabinoids, vol. 103, pp. 1–36, Jan. 2017, Springer.

[14] N. Happyana, S. Agnolet, R. Muntendam, A. Van Dam, B. Schneider, and O. Kayser, "Analysis of Cannabinoids in Laser-Microdissected Trichomes of Medicinal *Cannabis sativa* Using LCMS and Cryogenic NMR," Phytochemistry, vol. 87, pp. 51–59, Mar. 2013, doi: 10.1016/j.phytochem.2012.11.001.

[15] G. Grassi and J. M. McPartland, "Chemical and Morphological Phenotypes in Breeding of *Cannabis sativa* L.," *Cannabis sativa* L.-Botany and Biotechnology, pp. 137–160, May. 2017.

[16] F. Pellati, V. Borgonetti, V. Brighenti, M. Biagi, S. Benvenuti, and L. Corsi, "*Cannabis sativa* L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role Against Oxidative Stress, Inflammation, and Cancer," Biomed. Res. Int., vol. 12, no. 1691428, pp.1–15, Dec. 2018, doi: 10.1155/2018/1691428.

[17] P. Kumar, D. K. Mahato, M. Kamle, R. Borah, B. Sharma, S. Pandhi, V. Tripathi, H. S. Yadav, S. Devi, U. Patil, J. Xiao, and A. K. Mishra, "Pharmacological Properties, Therapeutic Potential, and Legal Status of *Cannabis sativa* L.: An overview. Phytother," Res., vol. 35, no. 11, pp. 6010–6029, Nov. 2021, doi: 10.1002/ptr.7213.

[18] K. S. Gould and C. Lister, "Flavonoid Functions in Plants," Flavonoids: Chemistry, Biochemistry and Applications, pp. 397–441, 2006.

[19] E. P. Baron, P. Lucas, J. Eades, and O. Hogue, "Patterns of Medicinal Cannabis Use, Strain Analysis, and Substitution Effect Among Patients with Migraine, Headache, Arthritis, and Chronic Pain in a Medicinal Cannabis Cohort," J. Headache Pain, vol. 19, no. 1, pp. 1–28, May. 2018, doi: 10.1186/s10194-018-0862-2.

[20] I. J. Flores-Sanchez and R. Verpoorte, "Secondary Metabolism in Cannabis," Phytochem. Rev., vol. 7, pp. 615–639, Apr. 2008, doi: 10.1007/s11101-008-9094-4.

[21] H. N. Matsuura and A. G. Fett-Neto, "Plant Alkaloids: Main Features, Toxicity, and Mechanisms of Action," Plant Toxin, vol. 2, no. 7, pp. 1–15, Jan. 2015, doi: 10.1007/978-94-007-6728-7_2-1.

[22] A. Onay, H. Yıldırım, and R. Ekinci, "Kenevir," Palme Yayınevi, 2020.

[23] E. Small, "Cannabis: A Complete Guide," CRC Press, Boca Raton, Jun. 2017.

[24] T. Serafimovska, M. Darkovska-Serafimovska, M. Mitevska, S. Stefanoski, Z. Keskovski, G. Stefkov, and J. Tonic Ribarska, "Determination of Terpenoid Profile in Dry Cannabis Flowers and Extracts Obtained from Different Cannabis Varieties," J. Pharm. Res. Int., vol. 33, no. 53B, pp. 214–228, Dec. 2021, doi:10.9734/JPRI/2021/v33i53B33698.

[25] S. Surendran, F. Qassadi, G. Surendran, D. Lilley, and M. Heinrich, "Myrcene–What are the Potential Health Benefits of This Flavouring and Aroma Agent?," Front Nutr., vol. 8, 699666, Jul. 2021, doi: 10.3389/fnut.2021.699666.

[26] D. Fiorini, A. Molle, M. Nabissi, G. Santini, G. Benelli and F. Maggi, "Valorizing Industrial Hemp (*Cannabis sativa* L.) By-Products: Cannabidiol Enrichment in the Inflorescence Essential Oil Optimizing Sample Pre-Treatment Prior to Distillation," Ind. Crops Prod., vol. 128, pp. 581–589, Feb. 2019, doi: 10.1016/j.indcrop.2018.10.045.

[27] S. P. S. Yadav, M. Kafle, N. P. Ghimire, N. K. Shah, P. Dahal, and S. Pokhrel, "An Overview of Phytochemical Constituents and Pharmacological Implications of *Cannabis sativa* L.," J. Herb. Med., vol. 42, 100798, Dec. 2023, doi: 10.1016/j.hermed.2023.100798.

[28] A. Janatová, I. Doskočil, M. Božik, A. Fraňková, P. Tlustoš, and P. Klouček, "The Chemical Composition of Ethanolic Extracts from Six Genotypes of Medical Cannabis (*Cannabis sativa* L.) and Their Selective Cytotoxic Activity," Chem. Biol. Interact, 353:109800, Feb. 2022, doi: 10.1016/j.cbi.2022.109800.

[29] A. Di Sotto, M. Gullì, A. Acquaviva, M. Tacchini, S. C. Di Simone, A. Chiavaroli, and C. Ferrante, "Phytochemical and Pharmacological Profiles of the Essential Oil from the Inflorescences of the *Cannabis sativa* L.," Ind. Crops Prod., vol. 183, 114980, Sep. 2022, doi: 10.1016/j.indcrop.2022.114980.

[30] D. Karou, W. M. Nadembega, L. Ouattara, D. P. Ilboudo, A. Canini, J. B. Nikiéma, and A. S. Traore, "African Ethnopharmacology and New Drug Discovery, " Med. Aromat. Plant Sci. Biotechnol., vol. 1, no. 1, pp. 61–69, 2007.

[31] A. E. Erdoğan, and A. Everest, "Antimikrobiyal Ajan Olarak Bitki Bileşenleri," Türk Bilimsel Derlemeler Dergisi, vol. 2, pp. 27–32, Jul. 2013.

[32] I. Galasso, R. Russo, S. Mapelli, E. Ponzoni, I. M. Brambilla, G. Battelli, and R. Reggiani, "Variability in Seed Traits in a Collection of *Cannabis sativa* L. Genotypes," Front Plant Sci., vol. 7, 7:688, May. 2016, doi: 10.3389/fpls.2016.00688.

[33] E. M. Ali, A. Z. Almagboul, S. M. Khogali, and U. M. Gergeir, "Antimicrobial Activity of *Cannabis sativa* L." Chin Med., vol. 3, no. 1, pp. 61–64, 2012, doi: 10.4236/cm.2012.31010.

[34] K. Ostapczuk, S. O. Apori, G. Estrada, and F. Tian, "Hemp Growth Factors and Extraction Methods Effect on Antimicrobial Activity of Hemp Seed Oil: A Systematic Review," Separations, vol. 8, no. 10, Oct. 2021, doi: 10.3390/separations8100183.

[35] F. Malik, S. Hussain, T. Mirza, A. Hameed, S. Ahmad, H. Riaz, P. A. Shah, and K. Usmanghani, "Screening for Antimicrobial Activity of Thirty-Three Medicinal Plants Used in the Traditional System of Medicine in Pakistan," J. Med. Plant Res., vol. 5, no. 14, pp. 3052–3060, Apr. 2011.

[36] R. Yasmeen, A. S. Hashmi, A. A. Anjum, S. Saeed, and K. Muhammad, "Antibacterial Activity of Indigenous Herbal Extracts Against Urease Producing Bacteria," J. Anim. Plant Sci., vol. 22, no. 2, pp. 416–419, 2012.

[37] A. Hazrat, M. Nisar and S. Zaman, "Antibacterial Activities of Sixteen Species of Medicinal Plants Reported from Dir Kohistan Valley KPK, Pakistan," Pak. J. Bot., vol. 45, no. 4, pp. 1369–1374, 2013.

[38] U. Iqbal, T. Mukhtar, and S. M. Iqbal, "In Vitro and In Vivo Evaluation of Antifungal Activities of Some Antagonistic Plants Against Charcoal Rot Causing Fungus Macrophomina Phaseolina," Pak. J. Agric. Sci., vol. 51, no. 3, pp. 691–696, 2014.

[39] M. Naveed, T. A. Khan, I. Ali, A. Hassan, H. Ali, Z. U. Din, Z. Hassan, S. Tabassum, M. A. Saqib, and M. U. Rehman, "In Vitro Antibacterial Activity of *Cannabis sativa* Leaf Extracts to Some Selective Pathogenicbacterial Strains," Int. J. Biosci., vol. 4, no. 4, pp. 65–70, Mar. 2014, doi: 10.12692/ijb/4.4.65-70.

[40] M. S. Isahq, M. S. Afridi, J. Ali, M. M. Hussain, S. Ahmad, and F. Kanwal, "Proximate Composition, Phytochemical Screening, GC-MS Studies of Biologically Active Cannabinoids and Antimicrobial Activities of *Cannabis indica*," Asian Pac. J. Trop. Dis., vol. 5, no. 11, pp. 897–902, 2015, doi: 10.1016/S2222-1808(15)60953-7.

[41] F. Rashid, F. A. Butt, S. Nasreen, F. U. Nisa, Z. Kanwal, A. Kaleem, and S. Andleeb, "In Vitro Antimicrobial and Antioxidant Activities of Two Medicinal Plants Against Some Clinically Important Bacteria," FUUAST J. Biol., vol. 6, no. 1, pp. 103–107, Jun. 2016.

[42] M. Anjum, Z. Arooj, S. Azam, P. Rehman, and J. Khadim, "Evaluation of Antimicrobial Activity and Ethnobotanical Study of *Cannabis sativa* L.," Pure Appl. Biol., vol. 7, no. 2, pp. 706–713, 2018, doi: 10.19045/bspab.2018.70088.

[43] S. Ullah, G. Jan, F. Gul, S. Khan, H. Husna, J. Sher, and S. Abidullah, "Phytochemistry and Antibacterial Activities of Some Selected Plants of War Affected Area of Bajaur Agency, Pakistan," J. Pharmacogn. Phytochem., vol. 7, no. 3, pp. 415–422, 2018.

[44] I. H. Khan and A. Javaid, "Antifungal Activity of Leaf Extract of *Cannabis sativa* against *Aspergillus flavipes*," Pak. J. Weed Sci. Res., vol. 26, no. 4, pp. 447–453, 2020, doi: 10.28941/pjwsr.v26i4.883.

[45] D. R. Rida and R. Batool, "Antibacterial and Antioxidant Characteristics of *Cannabis sativa*: A Medicinal Herb from Gilgit-Baltistan," Pak. J. Sci., vol. 72, no. 2, Jun. 2020, doi: 10.57041/pjs.v72i2.168.

[46] M. Zaka, S. S. Hashmi, M. A. Siddiqui, L. Rahman, S. Mushtaq, H. Ali, C. Hano, and B. H. Abbasi, "Callus-Mediated Biosynthesis of Ag and ZnO Nanoparticles Using Aqueous Callus Extract of *Cannabis sativa*: Their Cytotoxic Potential and Clinical Potential Against Human Pathogenic Bacteria and Fungi," Green Process Synthesis, vol. 10, no. 1, pp. 569–584, Sep. 2021, doi: 10.1515/gps-2021-0057.

[47] M. A. Blaskovich, A. M. Kavanagh, A. G. Elliott, B. Zhang, S. Ramu, M. Amado, and M. Thurn, "The Antimicrobial Potential of Cannabidiol," Commun. Biol., vol. 4, no. 1, pp. 1–18, Jan. 2021, doi: 10.1038/s42003-020-01530-y.

[48] S. A. Çelik and İ. Ayran, "Antioksidan Kaynağı Olarak Bazi Tıbbi ve Aromatik Bitkiler," Türk Bilimsel Derlemeler Dergisi, vol. 13, no. 2, pp. 115–125, 2020.

[49] G. Crescente, S. Piccolella, A. Esposito, M. Scognamiglio, A. Fiorentino, and S. Pacifico, "Chemical Composition and Nutraceutical Properties of Hempseed: An Ancient Food with Actual Functional Value," Phytochem. Rev., vol. 17, no. 4, pp. 733–749, Dec. 2018, doi: 10.1007/s11101-018-9556-2.

[50] E. E. Essien, "Effect of Extraction Conditions on Total Polyphenol Contents, Antioxidant and Antimicrobial Activities of *Cannabis sativa* L.," Elec. J. Env. Agricult. Food Chem., vol. 11, no. 4, pp. 300–307, 2011.

[51] F. Siano, S. Moccia, G. Picariello, G. L. Russo, G. Sorrentino, M. Di Stasio, and M. G. Volpe, "Comparative Study of Chemical, Biochemical Characteristic and ATR-FTIR Analysis of Seeds, Oil and Flour of the Edible Fedora Cultivar Hemp (*Cannabis sativa* L.)," Molecules, vol. 24, no. 1:83, Dec. 2018, doi: 10.3390/molecules24010083.

[52] F. Fathordoobady, A. Singh, D. D. Kitts, and A. P. Singh, "Hemp (*Cannabis sativa* L.) Extract: Anti-Microbial Properties, Methods of Extraction, and Potential Oral Delivery," Food Rev. Int., vol. 35, no. 7, pp. 664–684, Apr. 2019, doi: 10.1080/87559129.2019.1600539.

[53] S. Frassinetti, E. Moccia, L. Caltavuturo, M. Gabriele, V. Longo, L. Bellani, and L. Giorgetti, "Nutraceutical Potential of Hemp (*Cannabis sativa* L.) Seeds and Sprouts," Food Chem., vol. 262, pp. 56–66, Oct. 2018, doi: 10.1016/j.foodchem.2018.04.078.

[54] I. Nadeem, A. U. Khan, M. N. Ashar, M. Ashfaq, S. Shahid, and D. Ahmed, "In Vitro Total Antioxidant and Radical Scavenging Activities of Organic Extracts from Leaves, Stem and Inflorescence of *Cannabis sativa* L.," Asian J. Chem., vol. 24, no. 11, pp. 5067–5072, 2012.

[55] S. Naz, M. A. Hanif, H. N. Bhatti, and M. Shahid, "Partition, Fractionation, Antioxidant Potential and Phenolics Profiling of *Cannabis sativa* Growing in Pakistan," Oxid. Commun., vol. 39, no. 4, pp. 2946–2960, 2016.

[56] M. Ahmed, M. Ji, P. Qin, Z. Gu, Y. Liu, A. Sikandar, M. F. Iqbal, and A. Javeed, "Phytochemical Screening, Total Phenolic and Flavonoids Contents and Antioxidant Activities of *Citrullus colocynthis* L. and *Cannabis sativa* L.," Appl. Ecol. Environ. Res., vol. 17, no. 3, pp. 6961–6979, 2019, doi: 10.15666/aeer/1703_69616979.

[57] A. Ali, N. Akhtar, H. Khan, M. H. H. B. Asad, and Z. Ahmad, "The Improvement on the Skin Surface by a New Type of Dermocosmetic Loaded Plant Extract: A Split Face Skin Topographic Study," Pak. J. Pharm. Sci., vol. 33, no. 2, pp. 531–535, 2020, doi: 10.36721/PJPS.2020.33.2.REG.531-535.1.

[58] Rida, R. Dilshad, and R. Batool, "Antibacterial and Antioxidant Characteristics of *Cannabis sativa*: A Medicinal Herb From Gilgit-Baltistan," Pak. J. Sci., vol. 72, no. 2, Jun. 2020, doi: 10.57041/pjs.v72i2.168.

[59] S. A. Hussain, S.R. Abbas, S. M. Sabir, R. T. Khan, S. Ali, M. A. Nafees, S. W. Khan, A. Hussain, Q. Abbas, M. Ali, and S. A. E. Bukhari, "The Inhibitory Effect of *Cannabis sativa* L. and *Morus nigra* L. against Lipid Peroxidation in Goat Liver and Brain Homogenates," Braz. J. Biol., vol. 83, e247190, Oct. 2021, doi: 10.1590/1519-6984.247190.

[60] L. Izzo, L. Castaldo, A. Narváez, G. Graziani, A. Gaspari, Y. Rodríguez-Carrasco, and A. Ritieni, "Analysis of Phenolic Compounds in Commercial *Cannabis sativa* L. Inflorescences Using UHPLC-Q-Orbitrap HRMS," Molecules, vol. 25, no. 3, Jan. 2020, doi: 10.3390/molecules25030631.

[61] M. Pojic, A. Mišan, M. Sakac, T. Dapčević Hadnađev, B. Šarić, I. Milovanović, and M. Hadnađev, "Characterization of Byproducts Originating from Hemp Oil Processing," J. Agric. Food Chem., vol. 62, no. 51, pp. 12436–12442, 2014, doi: 10.1021/jf5044426.

[62] X. Ma, J. Yan, K. Xu, L. Guo, and H. Li, "Binding Mechanism of Trans-N-Caffeoyltyramine and Human Serum Albumin: Investigation by Multi-Spectroscopy and Docking Simulation," Bioorg. Chem., vol. 66, pp. 102–110, Jun. 2016, doi: 10.1016/j.bioorg.2016.04.002.

[63] G. Crescente, S. Piccolella, A. Esposito, M. Scognamiglio, A. Fiorentino, and S. Pacifico, "Chemical Composition and Nutraceutical Properties of Hempseed: An Ancient Food with Actual Functional Value," Phytochemistry Reviews, vol. 17, pp. 733–749, 2018, doi: 10.1007/s11101-018-9556-2.

[64] Y. Konca, T. Yüksel, S. Büyükkılıç Beyzi, and M. Özyürek, "Kanatlı Rasyonlarında Kenevir Tohumu Kullanılmasının Et ve Yumurta Yağ Asitleri Kompozisyonu Üzerine Etkileri," 12. Ulusal Zootekni Kongresi. 2016; 9-11 Mayıs, Isparta.

[65] J. Vitorović, N. Joković, N. Radulović, T. Mihajilov-Krstev, V. J. Cvetković, N. Jovanović, and N. Bernstein, "Antioxidant Activity of Hemp (*Cannabis sativa* L.) Seed Oil in *Drosophila melanogaster* Larvae Under Non-Stress and H_2O_2-Induced Oxidative Stress Conditions," Antioxidants, vol. 10, no. 6, 2021, doi: 10.3390/antiox10060830.

[66] Y. Xin, C. Schwarting, S. B. Armstrong, P. Nagib, E. E. Bonar, B. J. Arterberry, and A. K. Davis, "Increases in Cannabis Use and Negative Emotions During COVID-19 Pandemic Among College Students with Cannabis Use Disorder," J. Soc. Work Pract. Addict., vol. 24, no. 2, pp. 116–131, Aug. 2022, doi: 10.1080/1533256X.2022.2145067.

[67] Z. Waris, Y. Iqbal, A. Hussain, K. S. A. Shafqatullah, A. Ali, and M. W. Khan, "Proximate Composition, Phytochemical Analysis and Antioxidant Capacity of *Aloe vera*, *Cannabis sativa* and *Mentha longifolia*," Pure Appl. Biol., vol. 7, pp. 1122–1130, 2018.

[68] H. Nawaz, A. Nawaz, and A. Ahsan, "Total Phenolic Content and Antioxidant Potential of Local Varieties of Hemp in Pakistan (*Cannabis sativa*)," J. Plant Biochem. Physiol., vol. 9, no. 8, Oct. 2021.

[69] V. Menga, C. Garofalo, S. Suriano, R. Beleggia, S. A. Colecchia, D. Perrone, and C. Fares, "Phenolic Acid Composition and Antioxidant Activity of Whole and Defatted Seeds of Italian Hemp Cultivars: A Two-Year Case Study," Agriculture, vol. 12, no. 6, May. 2022, doi: 10.3390/agriculture12060759.

[70] R. Beleggia, V. Menga, F. Fulvio, C. Fares, and D. Trono, "Effect of Genotype, Year, and Their Interaction on the Accumulation of Bioactive Compounds and the Antioxidant Activity in Industrial Hemp (*Cannabis sativa* L.) Inflorescences," Int. J. Mol. Sci., vol. 24, no. 10, 2023, doi: 10.3390/ijms24108969.

[71] A. Hussain, S. H. Abidi, Q. Syed, and A. Saeed, "Current Knowledge on Ethnobotany, Phytochemistry and Biological Activities of Cannabis (hemp) from Pakistan with Emphasis on its Legalization and Regulation: Current Knowledge on Cannabis from Pakistan," Ethnobot. Res. Appl., vol. 23, pp. 1–33, 2022.

[72] A. Nafis, A. Kasrati, C. A. Jamali, N. Mezrioui, W. Setzer, A. Abbad, and L. Hassani, "Antioxidant Activity and Evidence for Synergism of *Cannabis sativa* (L.) Essential Oil with Antimicrobial Standards," Ind. Crops Prod., vol. 137, pp. 396–400, Oct. 2019, doi: 10.1016/j.indcrop.2019.05.032.

[73] A. André, M. Leupin, M. Kneubühl, V. Pedan, and I. Chetschik, "Evolution of the Polyphenol and Terpene Content, Antioxidant Activity and Plant Morphology of Eight Different Fiber-Type Cultivars of *Cannabis sativa* L. Cultivated at Three Sowing Densities," Plants, vol. 9, no. 12, Dec. 2020, doi: 10.3390/plants9121740.