

## Book Chapter

# Hemp Growth Factors and Extraction Methods Effect on Antimicrobial Activity of Hemp Seed Oil: A Systematic Review

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## Abstract

The bioactive Hemp Seed Oil (HSO) is becoming very popular in the medical and research fields due to its antimicrobial properties against several diseases caused by bacteria and fungi. However, the effect of hemp-growing factors and extraction methods on the bioactivity of HSO does not receive adequate research attention. Therefore, this review aims to investigate the effect of growth factors and extraction methods on the antimicrobial activity of HSO. Articles were retrieved from Google Scholar and the Scopus database and screened against inclusion and exclusion criteria. The study revealed that HSO prefers warm climates and favorable humidity ranging from 20 to 39 °C and 79–100% per year, respectively, and rainfall of 324 mm daily. The multivariate linear regression shown excellent prediction ( $R^2 = 0.94$ ) with climates upon Zone of Growth Inhibition (ZGI) of Gram-positive bacteria. Temperature is the strongest predictor ( $p < 0.01$ ) followed by humidity and rainfall ( $p < 0.05$ ). Furthermore, well-drained loam soil rich in organic matter seems to stimulate the antimicrobial activity of HSO. The major constituents that influence HSO's antimicrobial ability to *Staphylococcus aureus* were cannabidiol (CBD),  $\beta$ -caryophyllene, and limonene. The extraction methods showed less influence on the HSO bioactivity. HSO did not show significant antioxidant activity, but Hemp Seed Hull (HSH), Hemp Seed Flour (HSF), and Hydrolyzed Hemp Seed Protein (HPH), expressed promising DPPH scavenging ability.

## Keywords

Hemp Seed Oil (HSO); Antimicrobial; Zone of Growth Inhibition (ZGI); Rainfall; Temperature; Soil Type; Ex Traction Methods

## Abbreviations

HSO- Hemp Seed Oil; CBD- Cannabidiol; HSH- Hemp Seed Hull; HSF- Hemp Seed Flour; HPH- Hydrolyzed Hemp Seed Protein; DPPH-2,2-Diphenylpicrylhydrazyl; LA- Linoleic Acid; ALA- Alpha Linolenic Acid; PUFAs- Polyunsaturated Fatty Acids; GLA- Gamma Linoleic Acid; HEO- Hemp Essential Oil; THC- Tetrahydrocannabinol; ZGI- Zone of Growth Inhibition; MIC- Minimal Inhibition Concentration; CBG- Cannabigerol; HLE- Hemp Leaf Extract; BC- *Bacillus cereus*; BS- *Bacillus subtilis*; CF- *Citrobacter freundii*; ML- *Micrococcus luteus*; SA- *Staphylococcus aureus*; StE- *Staphylococcus epidermidis*; EC- *Escherichia coli*; EF- *Enterococcus faecalis*; SaE- *Salmonella enteritidis*; SeM- *Serratia marcescens*; PA- *Pseudomonas aeruginosa*; AN- *Aspergillus niger*; CA- *Candida albicans*; SP- *Streptococcus pneumoniae*; YE- *Yersinia enterocolitica*; CK- *Candida krusei*; CT- *Candida tropicalis*; ST- *Salmonella typhimurium*; StM- *Streptococcus mutans*; SC- *Saccharomyces cerevisiae*; KP- *Klebsiella pneumoniae*; DT- Distillation Time; mm- Millimeters; A- Acetone; M- Methanol; CBDA- Cannabidiolic Acid; ABTS-2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (biochemical reagent); HSK- Hemp Seed Kernel; SHR- Spontaneous Hypersensitive Rats; TAC- Total Antioxidant Capacity; SOD- Superoxide Dismutase; CAT- Catalase; T1- Trial One; T2- Trial Two; T3- Trial Three; T4- Trial Four

## Introduction

The hemp plant is considered one of the world's ancient cultivated plants that was developed from wild Cannabis plants originated from Central Asia and has long been widely used for textiles and as a nutritional source of food, cosmetics, and oriental medicine [1,2]. Hemp is also known as a short-day and

photoperiod-sensitive crop that exhibits several nutraceutical properties to produce cannabinoids, fatty acids, and other nutritional products for biomedical uses [3]. It is also an active ingredient in commercial insect repellents and biopesticides assigned to its antioxidant and antimicrobial properties [4,5]. Furthermore, hemp is a dioecious annual multipurpose crop grown for its seed, oil, food, and medicinal properties [6]. Hemp seed originates from the mature female *Cannabis sativa* L. plant constituting 25–35% lipid, 20–25% protein, and 30% carbohydrates, as reported by Deferne and Pate [7]. In addition, hemp seeds are used for the preparation of animal feed, oil, flour, and protein powder. In recent years, Hemp Seed Oil (HSO) has received public attention in its usage due to its valuable addition to human and animal diets. The hemp plant's seed oil contains anti-inflammatory properties due of its ideal ratio and high quantity of omega-6 (Linoleic Acid (LA)) to omega-3 (Alpha-Linolenic Acid (ALA)) Polyunsaturated Fatty Acids (PUFAs), 3:1, along with other PUFAs such as Gamma-Linoleic Acid (GLA) [8]. Furthermore, HSO possesses promising antimicrobial and antioxidant properties because of the plant's content in tocopherols (100–150 mg/100 g oil), terpenes, phenolic compounds, cannabinoids, vitamins, and minerals [9,10].

Out of all the plant's chemical compounds, cannabinoids represent the most studied group mainly because of their numerous pharmaceutical effects in humans [11]. More than 100 different chemical compounds called cannabinoids can be extracted from hemp as reported by García-Tejero et al. [12]. Many studies suggest that cannabidiol (CBD) has potential antimicrobial effects, especially against drug-resistant strains of *Staphylococcus aureus* [13–15]. Although HSO does not traditionally contain cannabinoids as it is found on the plant's leaves and flowers, an alternative hemp oil called Hemp Essential Oil (HEO) could be used to utilize a variety of the plant's beneficial components. HEO is defined as a complex mixture of several volatile compounds, mainly monoterpenes, sesquiterpenes, and other terpenoid-like substances [16]. It is extracted from the plant's leaves, flowers, seeds, seed bracts, inflorescences, and thinner stems [16]. Zheljzakov et al. [16]

found that HEO, containing the highest CBD, expressed the highest antimicrobial inhibition against Gram-positive bacteria. The two most abundant cannabinoids in the hemp plant are tetrahydrocannabinol (THC) and CBD, which are most popular for their therapeutic and psychotropic effects. The hemp plant contains 12–18% of CBD on average and 0.3% of THC, while the seed only contains a moderate amount of CBD and THC [17]. Fathordoobady et al. [18] concluded that the non-psychoactive cannabinoids such as CBD are present mainly in seed oil, while most THC accumulates in plant leaves. It is possible that the CBD presence in HSO could be caused by the hemp plant's contact with the resin secreted by the epidermal glands situated on flowers and leaves [19].

The chemical constituents of HSOs are tocopherols, terpenes, phenolic compounds, Vitamin B complex, sodium, calcium, iron, sulfur, potassium, phosphorus, zinc, and copper [2,20]. Due to the composition of these natural antioxidants in HSO, it was believed to have promising antioxidant effects. For example, the tocopherol acts as an antioxidant to prevent the oxidation of unsaturated fatty acids, as reported by Kriese et al. [21]. Unfortunately, at the end of this study, it was concluded that HSO did not perform as a good antioxidant; however, a more broad study would need to be completed in order to detect what influences the antioxidant activity in HSO.

The quality and the quantity of the bioactive components of the HSO depend on field conditions in which the hemp is being grown, the crop variety, and the extraction process used to obtain it [22]. The hemp's growth, yield, nutritional, and bioactive constituents depend on the interaction between the hemp variety, environment, and management [23]; here, plant density, soil fertility, and climatic conditions (rainfall, temperature, and humidity) are the main factors affecting hemp chemical compounds [24–26]. The interaction of these factors affects plant development, which will eventually result in low bioactive constituents of HSO. Togliatti's [27] study shows that growing conditions such as temperature, precipitation, and radiation affect hemp productivity. Poor soil and climatic conditions hinder the growth of hemp by restraining the root

development and nutrients constituents, especially when the soil is acidic and compacted [1]. On the contrary, many research findings indicated that hemp plants could survive harsh climatic conditions and poor soil environments. Therefore, some critical results regarding how the climatic conditions, soil environment, and bioactive compounds' extraction methods contribute to HSO's antimicrobial and antioxidant activity have not been explicit. Hence, this paper aims to elucidate these key gaps identified in the literature to provide an unambiguous end to these issues. Furthermore, this will give hemp cultivators a better understanding of the ideal temperatures, humidity, and water content that the plant needs for optimal nutritional benefits.

In this paper, we systematically review and organize the bioactivity of HSO literature toward the following objectives:

- To elucidate the effects of hemp's growth factors (temperature, rainfall, and humidity soil types) on the antimicrobial activity of hemp oils using existing evidence from peer-reviewed articles.
- To find out the various existing extraction methods on the antimicrobial activity of hemp oils.
- To provide the updated existing literature on the hemp-derived products' antimicrobial and antioxidant effects.

## **Materials and Methods**

### **Search Procedures**

HSO has been documented to contain bioactive properties. Many factors influence the components that express these properties, including extracting the oil, major constituents, and climate. The systematic review was gathered through a literature search from online databases. Relevant articles were searched on Google Scholar and the Scopus database to identify how climatic conditions and extraction methods affect Hemp Seed Oil's bioactivity. Boolean operators "AND" and "OR" were used to broaden the search. Some of the keywords used for searching were temperature AND bioactivity of HSO, antimicrobial AND antioxidant effects of HSO OR other hemp

oils, etc. The hemp location, temperature, rainfall, and humidity were researched [28]. The soil was identified through the Scopus database and Google Scholar online. Since the bioactivity of the HSO literature is so diverse, we supplemented our search with citations in recent studies (2004–2020). To further ensure that we had assembled a comprehensive list of studies, we asked researchers with the relevant knowledge on the topic to review and suggest keywords.

The search focused on scientific research articles using the following protocol:

- i. Publication years were between 2004 and 2020;
- ii. The keywords “hemp oil” AND “antimicrobial activity”; “hemp oil and climatic indicators”; hemp extraction method AND antimicrobial activity had to appear in the title and abstract;
- iii. They had to be scientific indexed papers only.

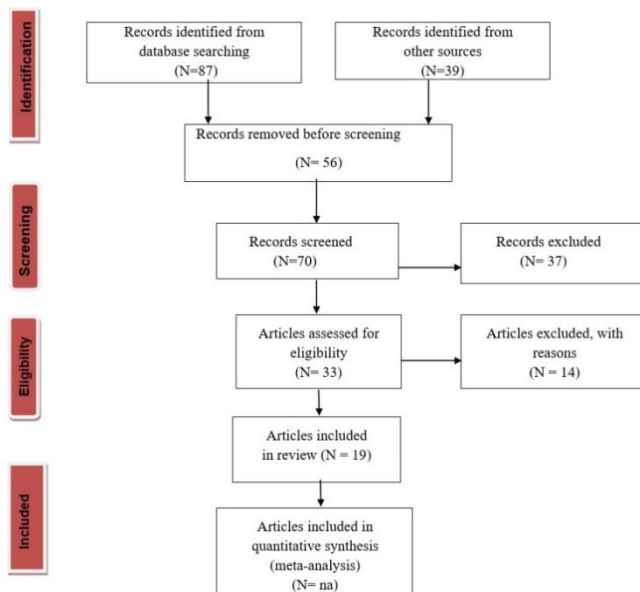
The results were screened against inclusion criteria i.e., articles that are not relevant to the studies. Full text of papers for all the articles that fitted into the inclusion criteria was retrieved.

## Screening

Strict criteria were used to determine the relevant articles for inclusion. For example, articles were being excluded if published in languages other than English or for which only an abstract was available, and then each remaining search result was grouped as one of the three publication types (criteria), which were adopted and modified by Gurwick et al. (2013) [26]:

- i. “Primary articles” Research papers appeared in the peer-reviewed literature and reported original data or results based on observations and experiments.
- ii. “Methods” papers evaluated or described an investigative technique for the extraction of chemical compounds of hemp oil.
- iii. “Review” papers summarized the understanding of bioactivity of hemp oil and antimicrobial activity [26].

Throughout the screening process, the number of publications excluded in each stage and their reasons for exclusion were noted based on the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement [29] in Figure 1.



**Figure 1:** PRISMA flow diagram for literature search; na = not applicable.

## Data Extraction and Reporting

A standard, purpose-designed form was designed to extract data (location, method of HSO extraction, antimicrobial activity, soil type and hemp product types) from the paper. Hemp antimicrobial activity is affected by the plant variety and growth stage of harvesting. However, none of the articles reported on the variety of hemp plant used, the type of fertilizer applied, and the period of harvesting of the hemp.

Nineteen papers were identified and reviewed on Google Scholar and Scopus for their antioxidant and antimicrobial properties. The articles retrieved range from 2004 to 2020. Eleven papers were compared to identify hemp oil's



antimicrobial activity. Four papers were based on HSO, three were based on HEO, two were based on cannabinoids in oil, and two were based on Hemp Leaf Extract (HLE).

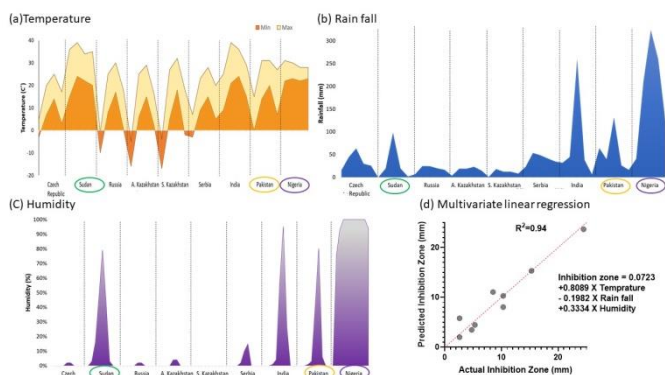
The oils were tested against the seven Gram-positive bacteria: *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Citrobacter freundii*, *Streptococcus pneumoniae*, *Serratia marcescens*, *Citrobacter freundii*, *Streptococcus mutans*, and *Staphylococcus epidermidis*; ten Gram-negative bacteria: *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Klebsiella pneumoniae*, and five fungi: *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Aspergillus niger*, and *Saccharomyces cerevisiae* testing for Zone of Growth Inhibition (ZGI), decrease in viable bacteria, and Minimal Inhibition Concentration (MIC). Seven papers out of the eleven gave eight locations: Europe (Czech Republic, Serbia, Russia), Asia (Russia, India, Kazakhstan, Pakistan), and Africa (Sudan and Nigeria).

Based on these eight locations, temperature, rainfall, humidity, and soil type were also analyzed. Temperature, rainfall, and humidity were investigated with multivariable linear regression. The average yearly/annual climate data were collected from WeatherSpark n.d. [28]. The average minimum and maximum temperature (°C) were taken from the months February, March, July, and October. Average rainfall (mm) was taken from the months February, March, July, October, and November using the formula:

$$\text{Average rainfall per month (mm)} = \frac{\text{lowest rainfall per month} + \text{highest rainfall per month}}{2} \quad (1)$$

The average humidity (%) of the start and end of each month was taken from the months February, March, July, October, and November. The relation between average temperature, rainfall, and humidity with the ZGI of Gram-positive bacteria was calculated via Excel (Figure 2d). Major constituents and extraction methods were also analyzed in regard to the antimicrobial potential of hemp products. Ten out of eleven

papers included the extraction method used to extract the oil from the plant. A table was put together to compare the antimicrobial potential and the extraction method used. Four papers out of eleven gave the plant's major constituents [16,30–32]. The antioxidant activity of HSO was observed through radical scavenging activity against 2,2-diphenylpicrylhydrazyl (DPPH). The radical scavenging activities of hemp oils and hemp products were investigated and were with each other, other oils, and their controls.



**Figure 2:** Climate upon inhibition zone. Average yearly (a) temperature (b), rainfall and (c) humidity of origin of hemp plant. Green circle: Highest against Gram-positive bacteria. Yellow circle: Highest against Gram-negative bacteria. Purple circle: Highest against fungi. (d) Multivariate linear regression. The Gram-positive bacteria were used in these analysis because HSO showed the most activity against Gram-positive bacteria. Figure 2d shows multivariate linear regression to find the relationship between the ZGI with temperature, rainfall, and humidity. The linear regression showed the climate on prediction of ZGI ( $R^2 = 0.94$ ). The prediction equation: Inhibition zone =  $0.0723 + 0.8089 \times \text{Temperature} - 0.1982 \times \text{Rainfall} + 0.3334 \times \text{Humidity}$ . Temperature is the strongest predictor ( $p < 0.01$ ) followed by humidity and rainfall ( $p < 0.05$ ).

## Result and Discussion

### Hemp-Derived Products' Major Constituents Influence on Antimicrobial Activity

For HSO to obtain high bioactive properties, it should be high in cannabinoids, monoterpenes, terpenes, tocopherols, and phenolic compounds. Eleven papers out of the total nineteen papers reviewed tested for hemp-derived products' antimicrobial effects and the summary of the antimicrobial effects of hemp-derived products is presented in Table 1.

Four out of these eleven papers gave the plant oils' major constituents, which were compared and observed to show which compounds performed the best in regard to antimicrobial activity. HSO and HEO were observed. The major components found in HSO were myrcene, b-sitosterol,  $\gamma$ -tocopherol, CBD,  $\beta$ -caryophyllene, and a trace amount of  $\alpha$ -tocopherol and methyl salicylate [32]. Other major constituents found in HEO were  $\alpha$ -caryophyllene, caryophyllene oxide, limonene,  $\beta$ -(E)-ocimene, and  $\alpha$ -pinene. More constituents are likely to be found in HEO rather than HSO because HEO contains various parts of the plant to produce the oil, such as the leaves, flowers, seeds, seed bracts, inflorescences, and thinner stems.

CBD is known to be a plant-derived cannabinoid (phytocannabinoid) that occurs uniquely in hemp plants [33]. In this current study, CBD, in particular, had a commonality within three of the papers that ranged between 0 and 52% in content. CBD, along with other cannabinoids, has been reported to be antibacterial, specifically against *Staphylococcus aureus* [13,15]. HEO from the 'wild Buro' and 'wild Saykaj' plants showed the most antimicrobial activity against all Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pneumoniae*) and Gram-negative bacteria (*Salmonella enterica*, *Pseudomonas aeruginosa*) compared to all other HEO, which contained less (Table 1). Similar studies conducted by Russo and Reggiani, 2013, concluded that the bioactive compounds in hemp plants are influenced by the hemp variety. However, none of the articles reported on the hemp age, variety, growth conditions (nutrition, humidity, and light levels), harvest time,

and storage conditions, making it difficult to deduce a proper conclusion [34].

Specifically, the CBD antimicrobial activity against Gram-negative bacteria depends on lipopolysaccharides (LPS) and the type or the species of the Gram-negative bacteria [16, Zhel CBD]. In addition, different types of LPS can be found in different genera of Gram-negative bacteria, contributing to 80% of the outer membrane of the Gram-negative bacteria. Therefore, the higher the lipopolysaccharides of the Gram-negative bacteria, the lower the possibility of CBD expressing its antimicrobial activities against the Gram-negative bacteria diseases or the higher the bacterial virulence [35]. HSO only expressed 10 mg/kg of CBD and did not exhibit much activity against Gram-negative bacteria due to the most abundant antigen (lipopolysaccharides) in the outer membrane of the most Gram-negative bacteria causing resistance to the CBD antimicrobial activity to inhibit the cell wall biosynthesis of the bacteria as well as the low amount of CBD contained in the HSO [36]. However, the amount of the CBD extracted from the HSO depends on the hemp age, variety, growth conditions (nutrition, humidity, and light levels), harvest time, and storage conditions, as reported by Sáez-Pérez et al. [22].

Moreover, oil derived from the hemp's seed did not exhibit any antifungal activity [9 Ali]. Whereas, oil extracted from the whole hemp plant did present moderate activity against fungi because of the high CBD, cannabigerol (CBG), and cannabichromene (CBC) present in the whole hemp plant oil [9]. Monoterpenes such as myrcene,  $\alpha$ -pinene, and limonene against *Escherichia coli*, *S. Enterica*, and *Staphylococcus aureus* have shown some antibacterial properties, which are most likely assigned to their diastereomeric structure, particularly limonene against Gram-positive bacteria [37, 38]. Limonene is most concentrated in HEO grown in India, ranging from 4.1 to 15.8%. The highest concentration of compounds (limonene,  $\alpha$ -pinene, terpinolene, myrcene, and  $\beta$ -(E)-ocimene) was observed in October, suggesting that the best harvest time to obtain antimicrobial properties is in October. Myrcene and  $\alpha$ -pinene are most concentrated in the HEO from Backi Petrovac, Serbia with 9.79–16.33% and 10.7–20.7% concentration [30].

Correspondingly,  $\beta$ -myrcene has shown antimicrobial potential in other studies [39,40]. All HEOs exhibited moderate to low activity against Gram-positive and Gram-negative bacteria.

**Table 1:** A Summary of Antimicrobial Effects of Hemp Derived Products.

Product Type	Method of Extraction	Method of Testing	Tested against:	Reference
HSO	Solvent extraction	Disk diffusion & Broth microdilution methods	BC, BS, ML, SA, StE, EC, CF, EF, SaE, SeM, PA	[4]
HSO	unknown	Well diffusion	SA, EC, PA, AN, SC	[32]
HSO & full-spectrum hemp	Cold pressed	Cold pressed seed oil soaked foil-backed acetate-based electrospun nanofibers tested for activity against SA.	SA	[41]
Seed Oil & Whole Plant	Solvent extraction	Cup plate agar diffusion method	BS, SA, EC, PA, AN, CA	[9]
HEO Registered Cultivars & Wild Hemp	Hydrodistillation	Disc diffusion method	SA, EF, SP, PA, SaE, YE, CA, CK, CT	[16]
HEO	Hydrodistillation	Disc diffusion method	SA, EF, SP, PA, SaE, YE, CA, CK, CT	[30]
HEO	Hydrodistillation	Filter paper disc diffusion assay	BS, SA, StM, EC, KP, PA, ST	[31]
CBD Oil (Flower)	Solvent extraction	MIC	SA, StE, EC, PA	[13]

Isolated cannabinoids THC, CBD, and CBG extracted from Flower	Solvent extraction	Well diffusion method	SA	[15]
HLE	Solvent extraction	Agar well diffusion method	SA, EC, PA	[42]
HLE	Solvent extraction	Disc diffusion method	SA, PA, CA, AN	[43]

Green: Gram positive bacteria; Yellow: Gram negative bacteria; Purple: Fungi.

Sesquiterpenes found in hemp oils such as  $\beta$ -caryophyllene, caryophyllene oxide,  $\alpha$ -humulene, and  $\delta$ -cadinene have also been reported to halt microbial growth [44,45]. ( $\beta$ -Caryophyllene has shown antibacterial activity against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Aspergillus niger* in the past [46], caryophyllene oxide against *Staphylococcus aureus* and *Enterococcus faecalis* [47],  $\alpha$ -humulene against *Bacillus cereus*, *Staphylococcus aureus*, and *Aspergillus niger* [45], and  $\delta$ -cadinene against *Bacillus subtilis*. Not only does  $\beta$ -caryophyllene show exceptional inhibitory results against bacteria and fungi, it also serves as a good antioxidant and anticarcinogenic terpene [46]. Zheljzakov et al. [16] exhibited the most  $\beta$ -caryophyllene out of 22.4–55%, which would correspond with its highest antimicrobial effect compared to the other three papers [16]. The result is similar to the findings of [48], as they concluded that the presence of sesquiterpenes or the presence of CBD could have an antimicrobial effect on the oil extracted from Cannabis Sativa.

Based on the Mikulcová et al. [4] study, it was concluded that the higher content of ALA could explain the higher antibacterial activity in unrefined oils compared to refined oils [4]. In addition, it was also proposed that because tocopherols and tetrahydrocannabinol are removed during the refining process,

they could indicate that they pertain to antimicrobial potentially in unrefined oils. It was also noted that Gram-positive bacteria were more sensitive to unrefined oils compared to Gram-negative strains because of Gram-positive bacteria's lack of a lipopolysaccharide outer layer. Gram-negative *Micrococcus luteus* and *Staphylococcus aureus* were proved the most sensitive to the oils, while *Escherichia coli* proved most resistant.

### **Climatic Indicators (Temperature, Rainfall, and Humidity) on Antimicrobial Activity of Hemp Seed Oil**

Figures 2a,b and c show that India, Sudan, Nigeria, and Pakistan express the highest temperatures, rainfall, and humidity levels. In relationship, the highest antimicrobial growth occurred in hemp oils sourced from Sudan, Nigeria, and Pakistan [9, 42,43]. Therefore, the rationale underpinning this objective is that if hemp plants experience optimal growing conditions of mild climates, humid atmosphere, and a rainfall of at least 635–762 mm per year, the plants will obtain high quantities of antimicrobial compounds. HSO cultivated in Niala, South Darfur, and Sudan possessed the highest activity against Gram-positive *Staphylococcus aureus* with 28 mm of ZGI. HSO did not show any activity against fungi, which may be due to the low bioactive component in the hemp seed, such as CBD CBG and CBC, causing low inhibition of ergosterol synthesis in fungi [9]. However, hemp oil derived from the whole hemp plant exhibited moderate antifungal activity against *Aspergillus niger* and *Candida albicans* due to their high CBD, CBG, and CBC, as reported by Ali et al. [9]. Its temperature was high, ranging from 15 to 39 °C (Figure 2a). The rainfall observed was higher in July (98 mm) (Figure 2b). Humidity increased from 0% in February to 79% in July, and it was 0% again in October (Figure 2c).

HSO from the hemp plant grown in Mansehra, Pakistan showed the most significant inhibitory effects against Gram-negative bacteria, particularly against *Escherichia coli* (22.2 mm) and *Pseudomonas aeruginosa* (25.3 mm). This may be due to the conducive growth condition (rainfall and fertile soil) of the hemp in the region, contributing to the high amount of CBD,

causing high inhibition of cell wall biosynthesis of the Gram-negative bacteria [9].

Pakistan's temperatures ranged from 0 °C in February to 31 °C in May and July (Figure 2a). The rainfall obtained by Pakistan was similar to the rainfall observed in Sudan, ranging from 16 to 131 mm a day (Figure 2b). Pakistan was the only location where the rainfall was high in February, decreased in May, and increased again in July (Figure 2b) [42]. The second most increased activity against Gram-negative bacteria was seen in HLE, in Nigeria, which may be assigned to the favourable growth conditions in the location needed for the hemp growth development. Mediavilla *et al.* [23] concluded that strong antimicrobial activity depends on tissue type, age, variety, growth conditions (nutrition, humidity, and light levels), harvest time, and storage conditions. However, none of the articles reported on the hemp age, variety, growth conditions (nutrition, humidity, and light levels), harvest time, and storage conditions, making it difficult to deduce a proper conclusion.

In Figure 2a–c, Nigeria possessed the highest climates, humidity, and rainfall compared to the other locations. The lowest Gram-negative bacteria activity was found in the Czech Republic, which was followed by India with only activity against *Salmonella typhimurium* and no activity in *Escherichia coli*, *K. Pneumoniae*, or *Pseudomonas aeruginosa*. Prague's temperatures ranged from –3 °C to 25 °C, rainfall ranged 15 mm to 64 mm a day, and humidity was only as high as 2%. The results obtained could be assigned to the possibility that the hemp plant was cultivated in the greenhouse, where temperature, humidity, and water content were highly ensured.

Nigeria obtained the highest temperature throughout the year, ranging from 22 °C to 31 °C, with the lowest and highest end of the range recorded by February and October. The temperature recorded in October was indicated as the highest compared to the other countries (Figure 2a). In addition to the high humidity of 93% to 100%, high rainfall of 40–324 mm daily, high soil fertility, and high content of CBD, the hemp leaf oil possessed the most increased antifungal activity up to 40 mm of growth inhibition against *Candida albicans*. The hemp plant that shows the highest activity against Gram-negative bacteria in Pakistan



was similar to the hemp plant grown in Nigeria [42,43]. The hemp plant was raised in an environment with high temperatures and humidity of 27 °C and 80%, respectively (Figure 2a,c). Both of these oils were extracts from leaves and showed similar results. However, the oil from Pakistan was not tested against fungi; therefore, it is unknown how much the climatic conditions affect the hemp plant's antifungal properties [42]. The leaf contains a large percentage of the plant's cannabinoids compared to all other parts of the plant [49].

Interestingly, even though climate intensities were lower in Pakistan, Pakistani HLE showed higher activity against Gram-negative bacteria [42]. According to Walsh *et al.* (2003), the potency of antimicrobial effects of hemp products depends on the type of seed and the part of the plant being used but not by the climatic indicators or soil, as had once been assumed [50].

India, Serbia, Kazakhstan, and Russia were all the sources of HEO, and all locations showed different antimicrobial effects. HEO sourced from India showed moderate to high antibacterial properties against Gram-positive bacteria and no activity against Gram-negative bacteria, apart from *Salmonella typhimurium*. Unlike the correlated results from Sudan, Pakistan, and Nigeria, HEO sourced from India obtained low antimicrobial effects despite the desirable climate between 9 and 39 °C in temperature, high rainfall between 6 and 260 mm, and humidity to 95%. The findings were due to the higher content of CBD in HLE compared to HEO. Both hemp plants grown in Pakistan and Nigeria were grown on garden soils and hemp plants in Sudan on woodland savanna/semi-arid soils, while Indian hemp was found on brown forest [31,42,43].

Serbian HEO showed lower antimicrobial activity. Its climate was significantly lower in all categories compared with Pakistan and Nigeria. HEO from Syrym Kazakhstan obtained the lowest temperatures, lowest rainfall, and lowest humidity levels throughout the year. Expectedly, it recorded the poorest antimicrobial activity, apart from *Candida albicans*, in Zheljzkov *et al.* [16]. These findings indicate that all three climate categories play a role in the antimicrobial activity of hemp plants and their oils. The three different HEO were sourced from Volgograd Oblast, Russia, with temperatures

ranging from  $-10$  to  $30$  °C, rainfall of 6–25 mm, and humidity of 0–2%. The rainfall and the temperature obtained are within the favorable climate conditions needed for hemp growth. Adesina et al. [3] reported that hemp grows best within a temperature range of  $16$  °C and  $27$  °C. The findings indicated that climatic conditions are not the only contributor to hemp oil's antibacterial and antifungal activity [3].

### Soil Type on the Effects of Antimicrobial Activity of Hemp Oils

The hemp plant can be grown on several types of soil. However, it seems to favour loose, deep, well-drained loam soils rich in organic matter with an optimum pH between 6.0 and 7.5 [51]. Soil preparation for hemp is similar to that of barley or spring wheat. For seed production, hemp is harvested when seeds begin to shatter [51,52]. Hemp plants grown in gardens seem to exhibit the best antimicrobial activity in Pakistan and Nigeria. The possible reason may be assigned to the daily maintenance of gardens: watering, pesticide control, soil amendment, and weed control. The agronomic performance influences the quality and quantity of the bioactive compounds, as reported by Adesina et al. [3].

HSO from Sudan showed high to moderate antibacterial activity, especially against *Staphylococcus aureus* and other Gram-positive bacteria. Sudan lies on a woodland savanna with semi-desert soils, which are also known as semi-arid soils. Semi-arid soils contain deep, dark, sandy clay, and loam and are mostly dry all growing season. In addition, semi-arid soils exhibit more organic matter whenever there is frequent rainfall and irrigation to supplement the rainfall deficit, as reported by Ros et al. [53]. Therefore, mostly in the semi-arid zones, hemp plants are usually grown under irrigated agriculture to increase the nutrient uptake for metabolic activities.

Chernozemic soils are described as black earth soil with high water-holding capacity that are calcium binding. They are humus-rich grassland soils with a pH of 7.2 and are commonly used for crop cultivation, including hemp plants in Backi Petrovac, Serbia [54]. HEO from Serbia possessed moderate antimicrobial activity; however, it was not as high as Russian

‘Buro’ and ‘Saykaj’ HEO sourced from Volgograd Oblast, Russia, with chernozemic and chestnut soils [16,30]. These two locations showed the highest antimicrobial activity against Gram-positive and Gram-negative bacteria in this study. They were both located on farmlands on opposite sides of the same river Volga. Both coordinates lead to a location near a small lake or swamp. ‘Titelski Greg’ HEO gave one of the poorest antimicrobial activities compared to other locations in this study. It was located in the same district as Saykaj, Mikhaylovsky District; however, it was not located near a lake, pond, or water source. The findings are clear indications that achieving the hemp plant water requirement will result in high antibacterial activity.

Humid soils located in Akzhaik and Syrym, Kazakhstan show moderate to low antimicrobial activity. Humid soils are deep and strongly weathered soils developed under aggressive, warm, and moist climates with high temperatures [55]. If the soil is too humid, it could promote pests, mold growth, and unwanted bacteria, altering the development of the hemp plant.

Lastly, hemp plants grown on forest soils or tarai soils showed moderate activity against Gram-positive and Gram-negative bacteria with little inhibition against fungi. These soils can either be rich with moisture from Artesan waters and swamps or drained from natural soil drains [56]. If the soil drains too rapidly, it will result in low water use efficiency of the hemp plant, as Larum (2020) reported [57]. According to Verma et al. [31], hemp plants cultivated on well-drained soil produce more antimicrobial properties, and HEO only had moderate activity against bacteria [31].

### **The Effects of Extraction Methods on Antimicrobial Effects of Hemp Oils**

Three different extraction methods were compared to identify which extraction method gives the best antimicrobial effects in the oil: solvent extraction, cold pressing, and hydrodistillation; see Table 2. Out of the three types of solvent extractions used in all eleven experiments, solvent extraction using methanol exhibited the most robust antimicrobial inhibition of 40 mm ZGI against fungi *Candida albicans* [43]. HSO in Ali et al. [9]

showed the second greatest ZGI of 28 mm against *Staphylococcus aureus* [9].

**Table 2:** Extraction methods on the effect on antimicrobial activity on hemp products.

Product Type	Method of Extraction	Antimicrobial Activity (mm)	Reference
Unrefined HSO	Solvent extraction (Methanol)	2.3–3.3, 0.3–3.0	[4]
HSO	Cold pressed	85.7–90.7%	[41]
HSO	Solvent extraction (Ethanol)	21, 28, 15, 16, n/a	[9]
HEO	Hydrodistillation	2–11, 1–12, 1–9	[16]
HEO	Hydrodistillation	2.50–7.17, 1–8.33, 3–13.8	[30]
HEO	Hydrodistillation	4–11, n/a–7	[31]
CBD Oil	Solvent extraction (Ethanol)	1–4 MIC $\mu$ g/mL, n/a	[13]
THC, CBD, and CBG	Solvent extraction (Acetone)	0.5–2 MIC $\mu$ g/mL	[15]
HLE	Solvent extraction (Ethanol)	10. 3, 22.2, 25.3	[42]
HLE	Solvent extraction (Methanol (M)) (Acetone (A))	A: 12, 16, 20 M: 10, 14, 20 A: 10, 10, 18 M: 11, 11, 18 A: 20, 25, 35 M: 25, 37, 40 n/a *	[43]

Green: G+ bacteria; Yellow: G- bacteria; Purple: Fungi Antimicrobial Activity; ZGI (mm), MIC (MIC $\mu$ g/mL), Decrease in viable bacteria (%) \* no effect on *Aspergillus niger*. Units in (mm). A: Acetone. M: Methanol. All the abbreviations of the microorganisms are indicated after the conclusion.

Mkpenie et al. [43] observed if the type of solvent used to extract the HSO, acetone or methanol, affected the antimicrobial properties of HSO. The ZGI of fungi showed 5–12 mm less in acetone than methanol extraction. The difference in acetone and methanol extraction against Gram-positive and Gram-negative bacteria did not show any significant difference. Unrefined HSO

was also extracted via methanol [4] and did not exhibit the same high result [9,42,43]. In addition, it had only 3 mm inhibition against *Staphylococcus aureus*, further confirming that the extraction method does not significantly influence antimicrobial events.

It is important to note that the temperature, rate of agitation, extraction time, and solvent to plant ratio all affect the final yield and antimicrobial potential of HSO [18]. Solvent extractions can be completed by a Soxhlet extractor or a DM technique [18]. However, ethanol seems to be the most popular choice of solvent used for extraction because it is considered adequate, efficient, and safe to handle [58,59], and methanol is better for the extraction of phytochemicals due to its polarity work [60].

Cold pressing as an extraction method has been conducted as a commonly used method due to its capacity to extract more phenolic compounds, chlorophyll, beta-carotene, flavonoids, gamma-tocopherols, alpha-tocopherols, and increase antioxidant capacity compared to solvent extractions [61,62]. However, the only disadvantage with cold pressing is that 60–80% of oil is out of the hemp seed [18 Fathordoobady]. Nevertheless, one study used a cold-pressing technique for HSO extraction and achieved an 85.7–90.7% decrease in viable bacteria against *Staphylococcus aureus* [41], suggesting promising results for cold-pressed HSO as an antimicrobial solution.

For traditional hydrodistilled methods, HEOs indicated moderate activity against bacteria and fungi [16,30,31]. There are three types of hydrodistillation techniques: water immersion, vapor immersion, and direct vapor immersion. The antimicrobial activity and major constituent extracted from the hydrodistilled oil are based on its Distillation Time (DT) and whether or not the hemp plant is grounded [30]. Hydrodistilled HEO showed promising results in CBD extractions of wild hemp plants and even pertained to a higher concentration of commercial hemp (7.5–7.8%) grown for CBD oil production [16]. Zheljaskov et al. [16] achieved up to 52.4% of CBD using hydrodistillation extraction, which was the highest recorded CBD content in a hemp plant using this extraction method, giving hydrodistillation a promising outlook for future tests [16].

**Table 3:** Antimicrobial effects of HSOs.

Header	BC	BS	ML	SA	CF	EF	EC	SaE	SeM	PA	CA	AN	SC	Reference
1	2.3/0	2.3/0	3.3/2.7	3/3	2.3/0	2.3/2.7	0.3/0	3/0	2.7/0.7	1.7/2.3				[4]
2				n/a			n/a					5 *	5 *	[32]
3				85.7– 90.7%										[41]
4		21		28 25 MIC			15 50 MIC			16 50 MIC	n/a	n/a		[9]

\* Zone of Growth Inhibition was not given, 5 mm was used as an estimate. Paper 2 showed some yeast inhibition during the first screening [32]. Units in (mm).

**Table 4.** Antimicrobial effects of HEOs.

	BS	SA	StM	EF	SP	EC	SaE	KP	PA	ST	YE	CA	CK	CT	Reference
5		2–11		2–8	3–10		2–11		3–8		1–10	3–9	1–8	2–7	[16]
6		3.25– 6.58		2.84– 7.17	2.5– 7.17		4.33– 8.33		1.67– 4.17		1– 8.17	3.83– 9.17	3–12	4.83– 13.8	[30]
7	4	10	9			n/a		n/a	n/a	7					[31]

HEO was extracted from leaves, flowers, seeds, seed bracts, inflorescences, and thinner stems. Units in (mm).

## Bacteria and Fungi Most Sensitive to HSO, HEO, Cannabinoids, and HLE (N = 11)

Tables 3 and 4 show the inhibition zones of HSO, HEO, cannabinoids, and HLE against Gram-positive bacteria, Gram-negative bacteria, and fungi.

### HSO

HSO showed the highest activity against Gram-positive bacteria, moderate activity against Gram-negative bacteria, and little to no activity against fungi (Table 3). The most sensitive bacteria to HSO was found to be *Staphylococcus aureus*, *Micrococcus luteus* followed by *Bacillus subtilis*. Ali et al. [9] showed the highest ZGI against *Staphylococcus aureus* out of all hemp-derived oils with an inhibition of 28 mm from the Cannabis seed oil itself and 12 mm in the whole plant. Inhibition of the seed oil was 8–16 mm greater than the gentamicin control and 16 mm greater than the whole plant [9]. The findings indicate that the content in the seed contains significant antibacterial effects against *Staphylococcus aureus* that are not found in the remainder of the plant. As a result of the effect of HSO on *Staphylococcus aureus*, HSO products can inhibit mainly skin infections such as acne and cellulitis, pneumonia, and bacteraemia. A likely reason HSO acts more deficient against Gram-negative bacteria is the outer protective lipopolysaccharide layer in the cell wall found in Gram-negative bacteria.

Nonetheless, HSO still expressed some inhibition against Gram-negative bacteria; therefore, it is a valuable oil to protect against bacteraemia, urinary tract infection, and pneumonia, often caused by *Escherichia coli* and *Pseudomonas aeruginosa*. However, more research should be conducted on HSO's ability to inhibit *Salmonella enteritidis*, *Serratia marcescens*, and *Micrococcus luteus*, since they gave higher inhibition rates in Mikulcová et al. [4] but were not tested against in Ali et al. [9]. In addition, HSO has been seen to inhibit yeast; however, it failed to replicate the results [32 Leizer].

## HEO

HEO showed low to moderate activity against all bacteria and fungi. *Staphylococcus aureus* was the most sensitive Gram-positive bacteria to HEO and *Pneumococcal pneumoniae* and *Streptococcus mutans*. *Staphylococcus aureus* was most sensitive against the ‘wild Buro’ HEO, containing 52.4% of CBD (Table 4). The other three Gram-positive bacteria experienced some but little inhibition, starting with the highest ZGI against *Pneumococcal pneumoniae*, *Enterococcus faecalis*, and *Bacillus subtilis*. Interestingly, HSO expressed high activity against *Bacillus subtilis* compared to HEO. However, HEO showed higher activity against fungi. Due to the HEO content, various parts of the plant may potentially exude antifungal properties from compounds found in different parts of the plant. The HEO use exhibited the highest antimicrobial activity against *Candida tropicalis* once grounded and distilled for 5–10 min, which was followed by *Candida albicans* and *Candida krusei*, which was not grounded at 0–5 DT. *Candida albicans* was also most sensitive to the ‘wild Saykaj’ oil, which is grown in Russia. In general, HEO showed lower antifungal properties in each control. Fluconazole’s inhibition against fungi averaged 3.5 to 15.7 mm higher than HEO in both papers. All bacterial inhibitions were lower than that of the control, suggesting that HEO is not an overly strong antimicrobial product.

## Cannabinoids and HLEs

Table 5 shows that cannabinoids, especially CBD, THC, and CBN, extracted from flowers showed lower MIC values to Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis*, which means that cannabinoids do not need to be present in high concentrations to exhibit antimicrobial effects. It has been reported that even the smallest amount of CBD or Cannabidiolic acid (CBDA) contamination in oil can positively impact Gram-positive bacterial inhibition [16,32]. Since the flower containing all the beneficial cannabinoids is located beside the seed, the flower could likely contaminate HSO during extraction, and this may explain why Ali et al. [9] obtained a significantly greater inhibition effect against Gram-positive



bacteria than Mikulcová et al. [4] and Leizer et al. [32]. Contrastingly, unlike Gram-positive bacteria, neither CBD nor CBDA showed any activity against Gram-negative bacteria, *Pseudomonas aeruginosa* or *Escherichia coli*, as reported by Martinenghi et al. [13], showing that cannabinoids work best against Gram-positive bacteria, and other major constituents in the oil cause inhibition against bacteria or fungi in HSO.

**Table 5:** Antimicrobial effects of cannabinoids and HLEs.

	SA	SE	EC	PA	CA	AN	Reference
8	1, 2–4 MIC	2–2 MIC	n/a	n/a			[13]
9	0.5–2 MIC						[15]
10	10.2		22.2	25.3			[42]
11	10–20			10–18	20–40	n/a	[43]

Papers 8, 9 represent cannabinoids [13,15]. Papers 10, 11 represent HLEs [42,43]. Units in (mm).

Next to cannabinoids, Table 5 shows HLEs, which also are a large source of cannabinoids such as CBD [63,64]. Once again, HLE showed high activity against *Staphylococcus aureus*; however, it possessed higher inhibition rates against Gram-negative bacteria and fungi. Inhibition of *Staphylococcus aureus* was similar but slightly higher than HEO; however, it was not as high as HSO inhibition, ranging from 10 to 20 mm ZGI [8,42,43,].

Compared to HSO and HEO, HLE experienced one of the highest inhibitory zones against Gram-negative bacteria, ranging from 10 mm to 25.3 mm ZGI. Mkpenie et al. [43] showed similar results for both Gram-positive and Gram-negative bacteria [43]. However, Naveed et al. [42] displayed 12–15 mm higher inhibitory effects against Gram-negative bacteria [42]. Furthermore, this study showed the highest amount of activity against *Escherichia coli* and *Pseudomonas aeruginosa*. According to Audu Sambo et al. [65], *Cannabis sativa* leaves contain larger quantities of amino acids, which would explain the higher antibacterial activity of HLE, since amino acids exhibit high antimicrobial effects.

Lastly, fungi experienced the greatest sensitivity to HLEs. It showed 20–40 mm ZGI against *Candida albicans*; however, it showed no activity against *Aspergillus niger*. This result would suggest why HEO expressed higher antifungal properties to HSO, as its ingredients include hemp leaves, unlike HSO. This was the highest ZGI overall, suggesting that the HLE is a good supplement to consume if suffering from Candidiasis. This study further proves that HSO is primarily effective against the inhibition of Gram-positive bacteria and that the antifungal properties of the plant are situated in other parts of the hemp plant, i.e., the leaf.

### Antioxidant Activity of Hemp Products

Many diseases are associated with the accumulation of free radicals. Therefore, to test for antioxidant activity, a plant can be tested for its scavenging activity against free radicals. DPPH, ABTS, and others are widely used as stable free radicals to observe the plant's scavenging abilities. All the papers tested for radical scavenging abilities against DPPH are presented in Table 6. Despite HSO's promising antioxidant components ( $\gamma$ -tocopherols, terpenes, and phenolic compounds), HSO still exhibited the lowest DPPH scavenging capabilities in three out of four research articles. HSO showed 0.43  $\mu\text{mol Trolox/mg}$  less scavenging ability to sunflower seeds [20 Senila], 49.7% less DPPH scavenging activity ascorbic acid, and 5.0–9.32% less DPPH activity compared with the male Wistar rats fed on a standard diet in vivo. HSO [10 Uluata] was the only one that did not show the least amount of DPPH scavenging ability compared to other oils. This cold-pressed HSO showed the second-highest scavenging ability, which was greater than that of terebinth, radish, stinging nettle, and laurel oil. HSO does not show a significant antioxidant ability because HSO is highly unstable because of the high content of unsaturated fatty acids [10 Uluata].

**Table 6:** Antioxidant activity against of HSO and hemp products.

Hemp Type	Origin	In Vivo/ In Vitro	Control	Radical Scavenging Activity against DPPH (Control)	Radical Scavenging Activity against DPPH	Reference
HSO	Romania	In vitro	Sunflower seeds	0.47 $\mu$ mol Trolox/mg sample	0.04 $\mu$ mol Trolox/mg sample	[20]
HSO	Turkey	In vitro	Laurel Oil Stinging nettle	85.79 mg Trolox/100 g oil 46.0 mg Trolox/100 g oil	62.37 mg Trolox/100 g oil	[10]
HSO	Los Angeles USA	In vitro	a-tocopherol ascorbic acid	86.3% DPPH 94.7% DPPH	45% DPPH	[66]
HSO, HSOP,WD PP	Italy	In vivo	Male Wistar rat fed with a standard diet	14.89–36.45% DPPH	5.57–31.39% DPPH	[5]
HSH, HSK	China	In vitro	Water	HSH: 2.21/1.09 DPPH IC50 mg/mL HSK: 2.15/4.55 DPPH IC50 mg/ml	HSH: 0.58 / 1.01 DPPH IC50 mg/mL HSK: 0.09 / 0.11 DPPH IC50 mg/ml	[67]
HSF	Canada	In vitro	Tea leaf and Rice flour crackers	40.22 DPPH ( $\mu$ mol TE/g d.w.)	7.47 DPPH ( $\mu$ mol TE/g d.w.)	[68]
HPH	Canada	In vitro	GSH	28%	HPH: 3%	[69]
HPH	Canada	In vitro	GSH	55%	HPH: 52%	[70]
		In vivo	SHR fed with casein-only diet	TAC: 0.145 mM/mL SOD: 81% CAT: 58% TPx (abs): 0.6	Trial 1, Trial 2, Trial 3 TAC: 0.2, 0.03, 0.21 mM/mL SOD: 90%, 87%, 98% CAT: 70%, 62%, 98% TPx (abs): 0.42, 0.49, 0.5	[70]

T1: (six-week-old SHR) fed four separate diets. Four rats out of eight were terminated after 8 weeks. T2: Remaining SHR were switched to regular chow diet for 4 weeks to serve as a washout period and allowed the establishment of oxidative stress. The now 20-week-old rats were fed four separate diet plans for 4 weeks and terminated. T3: (NTR) used 20-week-old rats fed three separate protein diets for 4 weeks. Then, they were terminated.

HSH, HSF, and HPH expressed slightly higher antioxidant activities based on their DPPH scavenging capabilities and may be assigned to the additional components found in the seed coat, resulting in higher antioxidant capacity. Chen et al. (2012) determined the IC<sub>50</sub> values of HSH and compared them to the HSK (seed without the hull) [67]. They concluded that HSH achieved a 0.92/0.42 lower IC<sub>50</sub> value than HSK, meaning that lower amounts of HSH will give the same antioxidant effect as HSH. Chen et al. (2012) further isolated two compounds that showed the most antioxidant activity: N-trans-caffeoyltyramine and cannabisin B [67]. Other studies have supported that these constituents possessed antioxidant abilities [71-73]. Furthermore, HSF also showed potential in antioxidant capabilities, because HSF is made from the shelled defatted hemp seed or the hull. It is already known from the previous study that HSH contains larger amounts of antioxidant components, making HSF a great additive for baked goods as a source of antioxidants.

HPH is a soluble form of hemp protein and is a hydrolyzed hemp protein isolate from defatted hemp flour. It is often used in skincare and as a dietary protein powder. Girgih et al. [69,70] conducted in vitro experiments on HPH to test their scavenging abilities against DPPH. The results found that HPH performed better against DPPH in the later study and showed great potential as an antioxidant. Girgih et al. [70] also conducted an in vivo experiment involving Hyper-Sensitive Rats (HSR) that were fed four different diets and showed that lipid peroxidation levels decreased 20-week-old SHR (T2) when compared to the young SHR (T1) [70]. The presence of HPH in the diets led to a significant ( $p < 0.05$ ) increase in plasma SOD and CAT levels in the T1 and T2 groups and a decrease in TPx levels. According to Girgih et al. [70], HPH contained antioxidant peptides that reduced the rate of lipid peroxidation in SHR with enhanced antioxidant enzyme levels and total antioxidant capacity [70].

To conclude the antioxidant activity in hemp seeds, the HSH around the kernel possessed the most antioxidant potential compared to HSO, which only uses the dehulled hemp seeds for oil production. The results suggest that to achieve optimal

antioxidant activity from the hemp seed, shelled hemp seed products such as HSF, HPH, or even hemp protein isolate (protein isolated from the hemp seeds) are needed.

Different parts of the plant in addition to HSO were utilized in this peer review, such as HSH, HPH, and HSF, due to the lack of papers focusing on the antioxidant properties of HSO alone. More studies should be conducted on the influence of antioxidant activity in HSO using the same method of testing and a variety of different sources of hemp plants.

## Conclusions

In this study, HSO exhibits antimicrobial activity based on its major constituents (cannabidiol (CBD),  $\beta$ -caryophyllene, and limonene). The growing factors influenced the bioactivity of the HSO such that humid soils showed low antimicrobial activity; in contrast, semi-arid soils revealed high antimicrobial activity against skin infections endocarditis and osteomyelitis caused by *Staphylococcus aureus*. HSO showed the highest activity against Gram-positive bacteria, specifically *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus subtilis*, but it was not limited to Gram-negative bacteria of *Escherichia coli* and *Pseudomonas aeruginosa*. In addition, three extraction methods (cold pressed, hydrodistillation, solvent extraction) are commonly used to extract HSO. However, the extraction methods have less influence on the HSO bioactivity. HSO worked best on Gram-positive bacteria, particularly *Staphylococcus aureus*, but other hemp-derived products with more CBD concentration such as Hemp Leaf Extract (HLE) performed better against fungi, particularly *Candida albicans*. In regard to the plant's growing conditions (temperature, humidity, rainfall), temperature exhibited the highest effect on inhibition levels against Gram-positive bacteria, followed by humidity and rainfall. HSO shows the greatest antibacterial ability against Gram-positive bacteria when grown in warm climates ranging from 20 to 39 °C. Furthermore, well-drained loam soils rich in organic matter seem to stimulate the HSO bioactivity. HSO did not show significant antioxidant activity; however, HSH, HSF, and HPH expressed promising DPPH scavenging ability. In conclusion,

HSO can be used in research for its antimicrobial purposes against Gram-positive bacteria. More research is needed to elucidate how limiting factors influence the bioactivity of hemp-derived product oils.

## References

1. Hayase S. Manila Hemp in World, Regional, National and Local History. *J. Asia-Pac. Stud.* 2018; 3: 171–181.
2. Li HL. An archaeological and historical account of cannabis in China. *Econ. Bot.* 1973; 28: 437–448.
3. Adesina I, Bhowmik A, Sharma H, Shahbazi A. A Review on the Current State of Knowledge of Growing Conditions, Agronomic Soil Health Practices and Utilities of Hemp in the United States. *Agriculture.* 2020; 10: 129.
4. Mikulcová V, Kašpárková V, Humpolíček P, Buňková L. Formulation, characterization and properties of hemp seed oil and its emulsions. *Molecules.* 2017; 22: 700.
5. Pasqua T, Rocca C, Lupi FR, Baldino N, Amelio D, et al. Cardiac and Metabolic Impact of Functional Foods with Antioxidant Properties Based on Whey Derived Proteins Enriched with Hemp Seed Oil. *Antioxidants.* 2020; 9: 1066.
6. Irakli M, Tsaliki E, Kalivas A, Kleisaris F, Sarrou E, et al. Effect of Genotype and Growing Year on the Nutritional, Phytochemical, and Antioxidant Properties of Industrial Hemp (*Cannabis sativa* L.) Seeds. *Antioxidants.* 2019; 8: 491.
7. Deferne JL, Pate DW. Hemp seed oil: A source of valuable essential fatty acids. *J. Int. Hemp. Assoc.* 1996; 3: 1–7.
8. Hazekamp A, Fishedick JT, Díez ML, Lubbe A, Ruhaak RL. Chemistry of cannabis. *Comprehensive natural products II.* Elsevier. 2010; 1033–1084.
9. Ali EMM, Almagboul AZI, Khogali SME, Gergeir UMA. Antimicrobial Activity of *Cannabis sativa* L. *Chin. Med.* 2012; 03: 61–64.
10. Uluata S, Özdemir N. Antioxidant Activities and Oxidative Stabilities of Some Unconventional Oilseeds. *J. Am. Oil Chem. Soc.* 2011; 89: 551–559.
11. Andre CM, Hausman JF, Guerriero G. *Cannabis sativa*: The Plant of the Thousand and One Molecules. *Front. Plant Sci.*

- 2016; 7: 19.
12. García-Tejero I, Zuazo VHD, Sánchez-Carnenero C, Hernández A, Ferreiro-Vera C, et al. Seeking suitable agronomical practices for industrial hemp (*Cannabis sativa* L.) cultivation for biomedical applications. *Ind. Crop. Prod.* 2019; 139: 111524.
  13. Martinenghi LD, Jönsson R, Lund T, Jenssen H. Isolation, Purification, and Antimicrobial Characterization of Cannabidiolic Acid and Cannabidiol from *Cannabis sativa* L. *Biomolecules.* 2020; 10: 900.
  14. Ferenczy L, Gracza L, Jakobey I. An antibacterial preparatum from hemp (*Cannabis sativa* L.). *Naturwissenschaften.* 1958; 45: 188–188.
  15. Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, et al. Antibacterial Cannabinoids from *Cannabis sativa*: A Structure–Activity Study. *J. Nat. Prod.* 2008; 71: 1427–1430.
  16. Zheljzakov VD, Sikora V, Dincheva I, Kačániová M, Astatkie T, et al. Industrial, CBD, and Wild Hemp: How Different Are Their Essential Oil Profile and Antimicrobial Activity? *Molecules.* 2020; 25: 4631.
  17. Murray D. CBD Oil vs. Hemp Seed Oil: How to Know What You’re Paying For. *Healthline.* 2020. Available online at: <https://www.healthline.com/health/hemp-vs-cbd-oil>
  18. Fathordoobady F, Singh A, Kitts DD, Pratap Singh A. Hemp (*Cannabis sativa* L.) extract: Antimicrobial properties, methods of extraction, and potential oral delivery. *Food Rev. Int.* 2019; 35: 664–684.
  19. Citti C, Pacchetti B, Vandelli MA, Forni F, Cannazza G. Analysis of cannabinoids in commercial hemp seed oil and decarboxylation kinetics studies of cannabidiolic acid (CBDA). *J. Pharm. Biomed. Anal.* 2018; 149: 532–540.
  20. Senila L, Neag E, Cadar O, Kovacs MH, Becze A, et al. Chemical, Nutritional and Antioxidant Characteristics of Different Food Seeds. *Appl. Sci.* 2020; 10: 1589.
  21. Kriese U, Schumann E, Weber W, Beyer M, Brühl L, et al. Oil content, tocopherol composition and fatty acid patterns of the seeds of 51 *Cannabis sativa* L. genotypes. *Euphytica.* 2004; 137: 339–351.
  22. Sáez-Pérez M, Brümmer M, Durán-Suárez J. A review of the

- factors affecting the properties and performance of hemp aggregate concretes. *J. Build. Eng.* 2020; 31: 101323.
23. Mediavilla V, Leupin M, Keller A. Influence of the growth stage of industrial hemp on the yield formation in relation to certain fibre quality traits. *Ind. Crop. Prod.* 2001; 13: 49–56.
  24. Amaducci S, Zatta A, Raffanini M, Venturi G. Characterisation of hemp (*Cannabis sativa* L.) roots under different growing conditions. *Plant. Soil.* 2008; 313: 227–235.
  25. Struik PC, Amaducci S, Bullard MJ, Stutterheim NC, Venturi G, et al. Agronomy of fibre hemp (*Cannabis sativa* L.) in Europe. *Ind. Crop. Prod.* 2000; 11: 107–118.
  26. Gurwick NP, Moore LA, Kelly C, Elias P. A Systematic Review of Biochar Research, with a Focus on Its Stability in situ and Its Promise as a Climate Mitigation Strategy. *PLoS ONE.* 2013; 8: e75932.
  27. Togliatti K, Archontoulis SV, Dietzel R, Puntel L, VanLoocke A. How does inclusion of weather forecasting impact in-season crop model predictions? *Field Crop. Res.* 2017; 214: 261–272.
  28. Weatherspark. Available online at: <https://weatherspark.com>
  29. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med.* 2009; 6: e1000097.
  30. Zheljzkov VD, Sikora V, Semerdjieva IB, Kačániová M, Astatkie T, et al. Grinding and Fractionation during Distillation Alter Hemp Essential Oil Profile and Its Antimicrobial Activity. *Molecules.* 2020; 25: 3943.
  31. Verma RS, Padalia RC, Verma SK, Chauhan A, Darokar MP. The essential oil of ‘bhang’ (*Cannabis sativa* L.) for non-narcotic applications. *Curr. Sci.* 2014; 107: 645–650.
  32. Leizer C, Ribnicky D, Poulev A, Dushenkov S, Raskin I. The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition. *J. Nutraceuticals Funct. Med. Foods.* 2000; 2: 35–53.
  33. Lambert DM, Fowler CJ. The Endocannabinoid System: Drug Targets, Lead Compounds, and Potential Therapeutic Applications. *J. Med. Chem.* 2005; 48: 5059–5087.
  34. Russo R, Reggiani R. Variability in Antinutritional



- Compounds in Hempseed Meal of Italian and French Varieties. *Plant*. 2013; 1: 25-29.
35. Blaskovich MAT, Kavanagh AM, Elliott AG, Zhang B, Ramu S, et al. The antimicrobial potential of cannabidiol. *Commun. Biol*. 2021; 4: 7.
  36. Raetz CRH, Ulevitch RI, Wright SD, Sibley CH, Ding A, et al. Gram-negative endotoxin: An extraordinary lipid with profound effects on eukaryotic signal transduction *FASEB J*. 1991; 5: 2652–2660.
  37. Sieniawska E, Swatko-Ossor M, Sawicki R, Skalicka-Woźniak K, Ginalska G. Natural Terpenes Influence the Activity of Antibiotics against Isolated Mycobacterium tuberculosis. *Med. Princ. Pract.* 2016; 26: 108–112.
  38. Wang CY, Chen YW, Hou CY. Antioxidant and antibacterial activity of seven predominant terpenoids. *Int. J. Food Prop.* 2019; 22: 230–238.
  39. Nasser AL-Jabri N, Hossain MA. Comparative chemical composition and antimicrobial activity study of essential oils from two imported lemon fruits samples against pathogenic bacteria. *Beni-Suef Univ. J. Basic Appl. Sci.* 2014; 3: 247–253.
  40. Zheljzkov VD, Kacaniova M, Dincheva I, Radoukova T, Semerdjieva IB, et al. Essential oil composition, antioxidant and antimicrobial activity of the galbuli of six juniper species. *Ind. Crop. Prod.* 2018; 124: 449–458.
  41. Wagner-Graham MA, Barndt H, Sunderland MA. Measurement of antibacterial properties of foil-backed electrospun nanofibers. *Fash. Text.* 2019; 6: 30.
  42. Naveed M, Khan TA, Ali I, Hassan A, Ali H, et al. In vitro antibacterial activity of Cannabis sativa leaf extracts to some selective pathogenicbacterial strains. *Int. J. Biosci.* 2014; 4: 65–70.
  43. Mkpennie VN, Essien EE, Udoh II. Effect of extraction conditions on total polyphenol contents, antioxidant and antimicrobial activities of Cannabis sativa L. *Electron. J. Environ. Agric. Food Chem.* 2012; 11: 300–307.
  44. Ali NAA, Chhetri BK, Dosoky NS, Shari K, Al-Fahad AJA, et al. Antimicrobial, Antioxidant, and Cytotoxic Activities of Ocimum forskolei and Teucrium yemense (Lamiaceae) Essential Oils. *Medicines*. 2017; 4: 17.

45. Schmidt JM, Noletto JA, Vogler B, Setzer WN. Abaco Bush Medicine: Chemical Composition of the Essential Oils of Four Aromatic Medicinal Plants from Abaco Island, Bahamas. *J. Herbs Spices Med. Plants.* 2007; 12: 43–65.
46. Dahham SS, Tabana YM, Iqbal MA, Ahamed MBK, Ezzat MO, et al. The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene  $\beta$ -Caryophyllene from the Essential Oil of *Aquilaria crassna*. *Molecules.* 2015; 20: 11808–11829.
47. Schmidt E, Bail S, Friedl SM, Jirovetz L, Buchbauer G, et al. Antimicrobial Activities of Single Aroma Compounds. *Nat. Prod. Commun.* 2010; 5: 1365–1368.
48. Novak J, Zitterl-Eglseer K, Deans SG, Franz CM. Essential oils of different cultivars of *Cannabis sativa* L. and their antimicrobial activity. *Flavour Fragr. J.* 2001; 16: 259–262.
49. Kleinhenz MD, Magnin G, Ensley SM, Griffin JJ, Goeser J, et al. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. *Appl. Anim. Sci.* 2020; 36: 489–494.
50. Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, et al. Activity and mechanisms of action of selected biocidal agents on Gram-positive and-negative bacteria. *J. Appl. Microbiol.* 2003; 94: 240–247.
51. Roseberg RJ, Jeliaskov VD, Angima SD. Soil, Seedbed Preparation and Seeding for Hemp’ OSU Oregon State University. 2019. Available online at: <https://catalog.extension.oregonstate.edu/em9239/html> (accessed on 2 June 2021)
52. Harper JK, Collins A, Kime L, Roth GW, Manzo HE. Industrial Hemp Production, PennState Extension. 2018. Available online at: <https://extension.psu.edu/industrial-hemp-production>
53. Ros M, Hernandez MT, García C. Soil microbial activity after restoration of a semi-arid soil by organic amendments. *Soil Biol. Biochem.* 2003, 35, 463–469.
54. Mihailović V, Čupina B, Hill GD, Mikić A, Świącicki W, et al. Grain yield components of white lupin lines grown on a chernozem soil in Serbia. In *México, Where Old and New World Lupins Meet, Proceedings of the 11th International Lupin Conference, Guadalajara, Mexico, 4–9 May 2005*;

- International Lupin Association, canterbury, New Zealand. 2006; 99–101.
55. Verheye W. Soils of the humid and sub-humid tropics. In Land Use, Land Cover and Soil Sciences—Volume VII: Soils and Soil Sciences-2. Oxford: EOLSS Publications. 2009; 7: 121.
56. Deshpande SB, Fehrenbacher JB, Ray BW. Mollisols of Tarai region of Uttar Pradesh, Northern India, Genesis and classification. *Geoderma*. 1971; 6: 195–201.
57. Larum D. What Does Well Drained Soil Mean: How To Get A Well-Drained Garden Soil. *Gardening Know How*. 2020. Available online at: <https://www.gardeningknowhow.com/garden-how-to/soil-fertilizers/what-is-well-drained-garden-soil.htm>
58. Brighenti V, Pellati F, Steinbach M, Maran D, Benvenuti S. Development of a new extraction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fibre-type *Cannabis sativa* L. (hemp). *J. Pharm. Biomed. Anal.* 2017; 143: 228–236.
59. Monroy YM, Rodrigues RA, Sartoratto A, Cabral FA. Extraction of bioactive compounds from cob and pericarp of purple corn (*Zea mays* L.) by sequential extraction in fixed bed extractor using supercritical CO<sub>2</sub>, ethanol, and water as solvents. *J. Supercrit. Fluids*. 2016; 107: 250–259.
60. Sidhu MC, Tanu Sharma. Antihyperglycemic activity of petroleum ether leaf extract of *Ficus krishnae* L. on alloxan-induced diabetic rats." *Indian journal of pharmaceutical sciences*. 2014; 76: 323.
61. The SS, Birch J. Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *J. Food Compos. Anal.* 2013; 30: 26–31.
62. Yilmaz E, Güneşer BA. Cold pressed versus solvent extracted lemon (*Citrus limon* L.) seed oils: Yield and properties. *J. Food Sci. Technol.* 2017; 54: 1891–1900.
63. Carus M, Sarmiento L. The European Hemp Industry: Cultivation, Processing and Applications for Fibres, Shives, Seeds and Flowers; European Industrial Hemp Association. Germany: Hürth. 2016; 1–9.
64. Vivek V. The Usages of Every Part of Hemp Plant' Hemp Foundation. 2019. Available online at:

- <https://hempfoundation.net/the-usages-of-every-part-of-hemp-plant/#:~:text=The%204%20parts%20of%20a,availability%20like%20cotton%20and%20petrol>
65. Audu Sambo B, Ofojekwu PC, Ujah A, Ajima M. Phytochemical, proximate composition, amino acid profile and characterization of Marijuana (*Cannabis sativa* L.) J. Phytopharm. 2014; 3: 35–43.
  66. Yu LL, Zhou KK, Parry J. Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils: Food Chemistry. Elsevier. 2005; 91: 723–729.
  67. Chen T, He J, Zhang J, Li X, Zhang H, et al. The isolation and identification of two compounds with predominant radical scavenging activity in hempseed (seed of *Cannabis sativa* L.). Food Chem. 2012; 134: 1030–1037.
  68. Radočaj O, Dimić E, Tsao R. Effects of hemp (*Cannabis sativa* L.) seed oil press-cake and decaffeinated green tea leaves (*Camellia sinensis*) on functional characteristics of gluten-free crackers. Journal of Food Science, Wiley Online Library. 2014; 79: C318–C325.
  69. Girgih AT, Udenigwe CC, Aluko RE. In vitro antioxidant properties of hemp seed (*Cannabis sativa* L.) protein hydrolysate fractions. J. Am. Oil Chem. Soc. 2011; 88: 381–389.
  70. Girgih AT, Alashi AM, He R, Malomo SA, Raj PD, et al. A Novel Hemp Seed Meal Protein Hydrolysate Reduces Oxidative Stress Factors in Spontaneously Hypertensive Rats. Nutrients. 2014; 6: 5652–5666.
  71. Al-Taweel AM, Perveen S, Muhammed El-Shafae A, Fawzy GA, Malik A, et al. Bioactive Phenolic Amides from *Celtis africana*. Molecules. 2012; 17: 2675–2682.
  72. Izzo L, Castaldo L, Narváez A, Graziani G, Gaspari A, et al. Analysis of phenolic compounds in commercial *Cannabis sativa* L. inflorescences using UHPLC-Q-Orbitrap HRMs. Molecules. 2020; 25: 631.
  73. Olatunji OJ, Chen H, Zhou Y. Neuroprotective effect of trans-N-caffeoyltyramine from *Lycium chinense* against H<sub>2</sub>O<sub>2</sub> induced cytotoxicity in PC12 cells by attenuating oxidative stress. Biomed. Pharmacother. 2017; 93: 895–902.