Current knowledge on ethnobotany, phytochemistry and biological activities of *Cannabis* (hemp) from Pakistan with emphasis on its legalization and regulation

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Review

Abstract

Background: Cannabis has a very extensive history of its uses as a medicinal plant that likely dates back more than two millennia. This review was envisioned to provide a brief summary on ethnobotany, phytochemistry, medicinal uses and some biological activities of *Cannabis* (hemp) with emphasis on its legalization and regulation in Pakistan.

Methods: The data on *Cannabis* was assembled from International scientific databases like Google Scholar, PubMed/Medline, Researchgate, SciELO, Scopus, Science Direct, Taylor and Francis, Web of Science, books, government reports, Master's and Ph.D. dissertations using specific keywords.

Results: In more than 33 different regions of Pakistan, the folk medicinal uses of *Cannabis* against ~60 ailments are still continuing. Phytochemistry data showed that more than 70 different compounds were reported in *Cannabis* from Pakistan with potential antioxidant activity. Overall, the antimicrobial activity reviewed here showed that *Cannabis* extracts against ~19 bacterial and 8 fungal strains possess potential inhibitory effects. Data on anticancer activity of *Cannabis* worldwide showed remarkable outcomes against more than 12 different cancer types and no data was found on the anticancer activity from Pakistan.

Conclusions: Conclusively, essential compounds isolated from *Cannabis* may exhibit different pharmacological actions and therefore support the utilization of species infusions and/or decoctions as folk traditional medicine in Pakistan. Through the legalization, revenue could be increased by exporting *Cannabis* based products or by exporting the raw material however, it should be complemented with extensive approaches to publicize the medicinal importance of *Cannabis* and appropriate policies should be developed for industrial and medicinal use.

Keywords: Cannabis, Ethnobotany, Phytochemistry, Biological activities, Legalization, Pakistan

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Background

The *Cannabis* genus from Cannabaceae family has both cultivated and wild forms with morphological variations leading some confusion in its taxonomy. A monotypic genus called *Cannabis sativa* has been anticipated by some authors while some argued that the genus *Cannabis* have two species, *Cannabis sativa* and *Cannabis indica*. A third species *Cannabis ruderalis* has been included as a third species in the genus by some authors (Hillig 2005). Species of *Cannabis* are very well adapted to various climate conditions from lower plains to high altitudes (10,000 ft). The origin of *Cannabis* is thought to be from Central Asia and now is widespread worldwide with significant recognition. Besides its wild occurrence, *Cannabis* is also cultivated in various regions of the world including Iran, China, India, Pakistan and Russia (Nasir *et al*. 2017). Over the centuries, *Cannabis* species are used as a source of food, fibers and oil. It is also employed as medicine and used for religious and recreational purposes (Piluzza *et al*. 2013). Its species like *C. sativa* possess a variety of active compounds like cannabinoids, alkaloids flavonoids and terpenoids (Andre *et al*. 2016). The most active compounds in greater amount are the cannabidiol (CBD). These are the class of terpenophenolic compounds of female flowers where they are accumulated largely in the trichome cavity (Taura *et al*. 2007). Out of the identified CBDs from *Cannabis* so far, the trans-Δ-9-tetrahydrocannabinol (D9-THC) is significant due to its psychoactive properties (Atakan 2012; Whiting *et al*. 2015) having the feelings of euphoria or pleasure to the user. The early humans with sedentary standards of living were the first to cultivate *C. sativa* (Russo 2007). Initially, it was an agricultural crop cultivated in China which then transferred to Africa, Middle East, and Asia where it was used as analgesic and for the treatment of malaria, gout, poor memory, and Rheumatism and also recreational uses were common (Kalant 2001). According to UNODC (2016), there are projected 182.5 million users of *Cannabis* globally and is extensively cultivated, marketed and used up in the form of drug. The recent *C. sativa* varieties with low amount of psychoactive D9-THC are essentially used for making fish nets ropes, strings, paper and textiles (Sabo *et al*. 2013).

Medicinal uses of *Cannabis* have been reported globally (Kalant 2001; Ahmad *et al*. 2006; Ibrar *et al*. 2007; Hussain *et al*. 2010; Ahmad *et al*. 2011; Barkatullah and Ibrar 2011; Haq *et al*. 2011; Iqbal *et al*. 2011; Ahmad *et al*. 2012; Levinsohn & Hill 2020; Laczkovics *et al*. 2021) especially from the greater Himalayan regions including Kashmir (Mir *et al*. 2021; Jan *et al*. 2021; Jan *et al*. 2022a, b; Mir *et al*. 2022). According to Mir *et al*. (2021), the dried leaves of *C. sativa* are smoked through a pipe called *Hukkah* in Kashmiri to get relief from depression and its leaf extract is used against cholera. Jan *et al*. (2021) reported the uses of *C. sativa* leaf infusion against labor pain during pregnancy from the Baramulla District of Jammu and Kashmir.

Another study (Jan *et al*. 2022a) from the same region evaluated the medicinal plants used for the treatment of various gynecological problems where *C. sativa has* the highest UV and RFC values that could be valuable for the discovery of novel drugs for female reproductive healthcare.

The dried buds and flowers of female *Cannabis* are consumed as a medicinal remedy in many countries. Its resin is limited in some countries like Southwest Asia, North Africa, and Middle East. *Cannabis* in the streets is found in the form of small stems or leaves (bhang, marijuana, kif or dagga), as a resin (hash, hashish, polm, charas), female flower heads (sensimilla) or oil (alcoholic resin extract). All these forms of *Cannabis* have varying purity levels with 1 to 60% of psychoactive compound like THC (Cunha-Oliveira *et al*. 2013). In 2011, Afghanistan, Morocco, Lebanon, India, and Pakistan were the main countries listed as source countries of 'hashish'. Between 2009 and 2011, Morocco and Afghanistan were the origin of 'hashish', and they reflect the main markets of *Cannabis* as Morocco and resin as Afghanistan. Morocco was the source of hashish smuggled from 17 different countries and of the 17 countries, 11 are in Central and Western Europe. The Middle East and European countries also designated Afghanistan as a 'hashish' seizures source.

Afghanistan as the main source of 'hashish' has also been reflected by the global distribution. During 2000 to 2011, Spain was dominated as global *Cannabis* resin seizures that are the main entry point for *Cannabis* resin in Europe from Morocco. According to UNODC (2013) in 2011, Spain accounted for 34 % of higher global seizures, and Pakistan and Morocco counted 18 and 12 % of global seizures. *Cannabis* was listed as Schedule 1 and 4 drugs in 1970 as having no accepted medicinal uses; therefore it was also restricted for scientific studies previously. Later, its medicinal significance was realized with fewer side effects as compared to previous data on *Cannabis* (Kalant 2001). In April 2015 in about 23 states of USA, due to the medicinal significance, *Cannabis* for medical purposes was legalized. More countries like Germany, Holland, France, Spain, Canada, Czech Republic and Colombia have legalized *Cannabis* use due to its therapeutic possessions for the treatment of multiple sclerosis, anorexia, neurodegenerative disorders, glaucoma, epilepsy, osteoporosis, cardiovascular disorders, schizophrenia, metabolic syndrome-related disorders and cancer (Kogan & Mechoulam 2007). Due to the significant medicinal properties,

the UN has decided to exclude *Cannabis* from Schedule 4 list and after decades of control, opened the market for *Cannabis*. According to a study, after exclusion of *Cannabis* from Schedule 4 in the USA, 17% marijuana users reported its medicinal uses and effectiveness (Morris *et al*. 2014) against various health problems and diseases with growing literature deciphering the therapeutic potentials of this plant (Freeman *et al*. 2019). Countries where *Cannabis* has been legalized are thriving hard to report on more potential medicinal aspects of *Cannabis*. Keeping in mind the potential health benefits of *Cannabis*, the present review aims to provide a comprehensive assessment from the old times to the recent days on the ethno-botanical, phytochemical and biological activities of *Cannabis* specifically from Pakistan. This review helps in promoting further research on the pharmaceutical aspects of *Cannabis* to uncover its usefulness as a therapeutic agent and also emphasize its recent legalization in Pakistan.

Materials and Methods

Search approach

This review was accomplished by going through an extensive detailed investigations on ethnopharmacological applications, phytochemistry and biological activities of *Cannabis* from Pakistan by using International scientific databases like Google Scholar, Pubmed/Medline, Researchgate, SciELO, Scopus, Science Direct, Springer Link, Taylor and Francis, Web of Science, books, government reports, Master's and Ph.D. dissertations using specific keywords like *Cannabis* from Pakistan, THC, CBD, *Cannabis* ethnobotany, *Cannabis* phytochemistry, *Cannabis* antibacterial activity, *Cannabis* antifungal activity, and *Cannabis* anticancer activity. The data on *Cannabis* was collected from published research articles up to February 2022 in English language. All the retrieved articles deciphering the ethnobotanical, phytochemical and information on biological activities of *Cannabis* from Pakistan were counted in (Fig. 1). Articles with unsatisfactory substantiation were excluded from the review. The reference lists of articles included were also checked to extract more relevant data on *Cannabis* from Pakistan to avoid loss of articles with appropriate information. The remaining articles were scrutinized to pick the appropriate articles and full texts of the articles were checked analytically for the extraction of data.

Results

A total of 132 titles or abstract were reviewed by the search strategy and 92 papers were included in the current review published on *Cannabis* from Pakistan (Fig. 1). The details of selected studies on *Cannabis* ethnobotany, phytochemistry and biological activities and its legalization and regulation in Pakistan are presented in Tables 1-4. Based on the collected data of this review, it was found that in more than 33 regions of Pakistan, still the uses of *Cannabis* as traditional medicine against ~60 health related problems continue (Table 1). Phyto-chemistry data showed that more than 70 different compounds (Few of them are shown in Fig. 2) were reported from the Pakistani *Cannabis* species (Table 2).

The antimicrobial activity of Pakistani *Cannabis* extracts screened against ~19 bacterial strains and 8 fungal strains with remarkable activity against the tested strains (Table 3). The antioxidant activity of Pakistani *Cannabis* species using azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS), 2,2-Diphenyl, 1-picrylhydrazyl hydrate (DPPH), Trolox equivalent antioxidant capacity (TAEC) methods revealed significant activity as shown in Table 4. A very limited data on anticancer activities of Pakistani *Cannabis* from some regions of Pakistan have been documented so far.

Medicinal uses of Cannabidiol (CBD) from *Cannabis*

In the recent past, FDA in its document highlighted some of the *Cannabis* derived compounds with potential therapeutic effects against various diseases. However the *Cannabis* marketing application has not been approved yet by the FDA, but three synthetic drug products from *Cannabis* are approved that contain nabilone or dronabinol and the Cannabidiol. According to (Todaro 2012), among the cannabinoid medications, dronabinol and nabilone were originally permitted in 1985 Ginat vomiting, and nausea linked with cancer chemotherapy in those patients who were unable to respond sufficiently to the conventional antiemetic cures. Presently, the CBD from *Cannabis* is the only approved drug for children suffering from Dravet syndrome and Lennox-Gastaut syndrome which are the seizure disorders (Silvestro *et al*. 2019). In 2018, CBD was approved by the FDA, and till now it is the only appropriate cure for the Dravet syndrome patients (Lattanzi *et al*. 2020). The particular pathophysiology is not known yet. As a first line treatment, numerous anti-epileptic drugs with CBD are given as a therapy. Lennox-Gastaut syndrome in children have usually intellectual and learning incapacities (Wirell 2016; Asadi-Pooya 2018).

The cannabinoid type-1 (CB_1) receptors are found in the neuromuscular and mucosa layers of the colon which are also expressed in the plasma cells and thereby influencing the inflammation of mucosa (Wright *et al*. 2005). The endo-cannabinoids like CBDs activity on CB_1 receptors in animal models while irritable bowel syndrome showed inhibition of colonic propulsion and small intestinal and gastric transit (Pinto *et al*. 2002). Studies have shown the

effects of endo-cannabinoids on colonic motility and gastric motility (Esfandyari *et al.* 2006). Therefore, CBDs have the potential to provide therapeutic effects against irritable bowel syndrome (Wong *et al.* 2012). The FDA approved CBD containing drug is also investigated against various neurodegenerative, psychiatric, cancerous, and inflammatory diseases (Levinsohn & Hill 2020; Laczkovics *et al.* 2021).

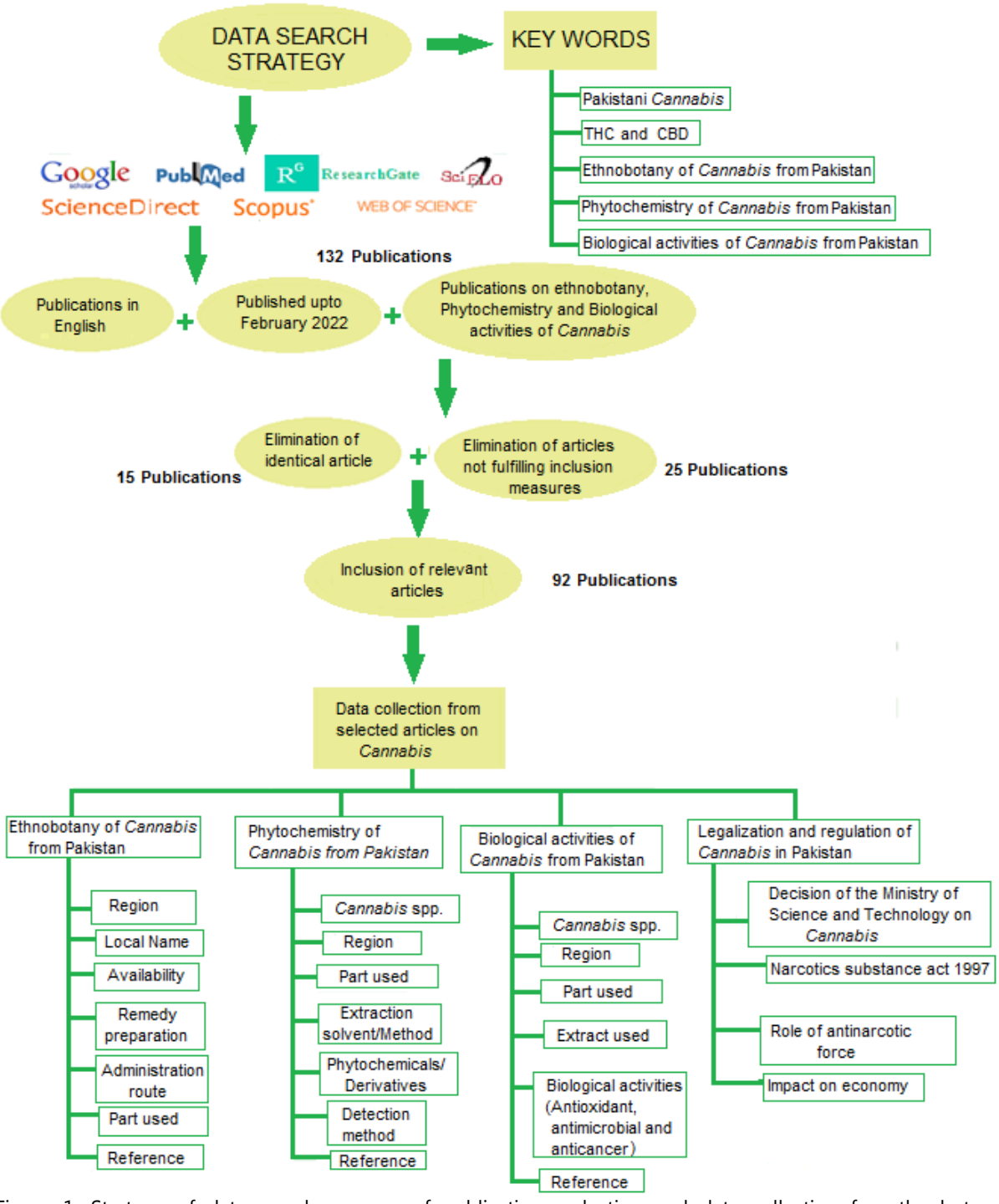


Figure 1. Strategy of data search, process of publications selection and data collection for ethnobotany, phytochemistry, biological activities and legalization of *Cannabis* in Pakistan.

CBD could be also suggested for the treatment of epilepsy which affects people across all age ranges. Despite of the availability of many antiepileptic medications in the market, the epilepsy in about one-third of persons is still continuing to have seizures even after treatment with those drugs (Mohanraj & Brodie 2006). In this regard, both the CBD and THC from *Cannabis* in animal models were shown to prevent seizures (Devinsky *et al.* 2014). Some

clinical investigations also checked the activity of CBD in mood disorders including control for chronic pain, anxiety, Alzheimer, antitumorigenic and anti-inflammatory attributes. Other diseases where CBD has been assessed include motor system symptoms associated with parkinson's disease, huntington's disease (Consroe *et al*. 1991; Pazos *et al*. 2008; Curtis *et al*. 2009; Koppel *et al*. 2014), multiple sclerosis (Whiting *et al*. 2015), dementia (Grundy 2002; Baker *et al*. 2003), Galucoma (Whiting *et al*. 2015; Prum *et al*. 2016) and sleep disorders (Garcia & Salloum 2015). Nevertheless, FDA has not yet approved the use of CBD to treat these diseases (Crippa *et al*. 2018; Watt & Karl 2017).

Cannabis species in Pakistan

The genus *Cannabis* includes three major species identified globally i.e. *C. sativa, C. indica* and C. *ruderalis*. The concentrations of CBD and THC vary among the species where, *C. sativa* possess highest concentration of THC and lowest concentration of CBD (Singh *et al*. 2018). According to the Flora of Pakistan, Cannabaceae is a small family with 2 genera and 3 species. These are mostly found in the northern temperate regions of the world and present in the Western part of Pakistan. Two species namely, *C. sativa* and *C. indica* of the genus *Cannabis* are widely distributed in the country.

According to the 1983 data of Pakistan Narcotics Control Board (PNCB), the usage of drug was stable in the 1950s to1970s with *Cannabis* and *Opium* were common, nevertheless in the late 1960s and early 1970s, an increase in *Cannabis* usage was noticed among youngsters due to the Western pop culture influence. In Pakistan, *Cannabis* is the most frequently used drug with 3.6 % prevalence in the population equal to 4 million users across the country (UNODC 2013). It is extensively consumed as a drink (bhang) and smoked as charas or hashish. Conversely, Pakistan's research community still thrives hard to bring to light both beneficial and adverse effects of *Cannabis.*

Ethnobotany of _Cannabis_ from Pakistan

Since the antiquity, medicinal plants from different genera have extraordinary applications and has traditionally been utilized against a number of diseases (Petrovska 2012). One of the most frequently used plants known to humans for thousands of years is *Cannabis* with ancient traditional uses history worldwide. In China, the oldest record of its medicinal uses known dates back to 4700 B.P. Several other oldest texts from Egypt, Persia, India, Rome and Greece also encompass valuable data about other medicinal uses of *Cannabis* medicinal (Abel 1980).

The folk traditional medicinal uses of *Cannabis* (Table 1) have been evaluated by various researchers from different regions of Pakistan (Ahmad *et al*. 2006; Ibrar *et al*. 2007; Hussain *et al*. 2010; Ahmad *et al*. 2011; Barkatullah and Ibrar 2011; Haq *et al*. 2011; Iqbal *et al*. 2011; Ahmad *et al*. 2012; Awan *et al*. 2013; Khan *et al*. 2013; Ajaib *et al*. 2014; Bibi *et al*. 2014; Shuaib *et al*. 2014; Jabeen *et al*. 2015; Ali *et al*. 2016; Aziz *et al*. 2016; Samreen *et al*. 2016; Shah *et al*. 2016; Umair *et al*. 2017; Amin *et al*. 2018; Hussain *et al*. 2018; Khan *et al*. 2018; Khan *et al*. 2018; Abbas *et al*. 2019; Rahman *et al*. 2019; Shah *et al*. 2019; Ali *et al*. 2020: Amjad *et al*. 2020; Rehman *et al*. 2020; Sulaiman *et al*. 2020; Akhtar *et al*. 2021; Iqbal *et al*. 2021; Hussain *et al*. 2022 and references therein).

Data on the ethnobotany of *Cannabis* from Pakistan for this review validated that maximum number of ethnobotanical studies on *Cannabis* were reported from the Khyber Pukhtunkhwa province (52%) followed by Punjab (27%), Gilgit-Baltistan (12%), Azad Jammu and Kashmir (6%) and Balochistan (3%). *Cannabis* in these regions is found in wild form and its remedies are mostly prepared as decoction, powder, juice, paste or infusions. The region wise ethnobotany of *Cannabis* as distributed in the country is shown in Fig. 2.

In the KPK province, the native people of Booni valley of Chitral use *Cannabis* leaves, seeds and floral buds as folk medicine to treat jaundice, bronchitis and malaria (Ahmed *et al*. 2006). In the Ranyal hills of Shangla, *Cannabis* has been employed as sedative and narcotic (Ibrar *et al*. 2007). Ahmed *et al*. (2011) conveyed the traditional uses of *Cannabis* from the Kabal Swat, where juice from the leaves of *C. sativa* has been used as antimalarial remedy and pain killer. Uses of C. sativa leaves against colic pain and male impotency and making in hashish (chars) were also reported. Barkatullah & Ibrar (2011) documented traditional uses of *C. sativa* shoots as hypnotic, narcotic and sedative from the Malakand.

In a related study, Haq *et al*. (2011) validated *C. sativa* leaves as sedative, anodyne and narcotic from Battagram. From the lower Dir, Shuaib *et al*. (2014) reported folk uses of *C. sativa* leaves as diuretic and stimulant. It has been validated that a mixture of leaves sugar and water is used against inflammation of stomach, indigestion and liver problems. A special remedy called tandai is prepared in Dir region from the *C. sativa* plant that helps in keeping the body cool during summer.

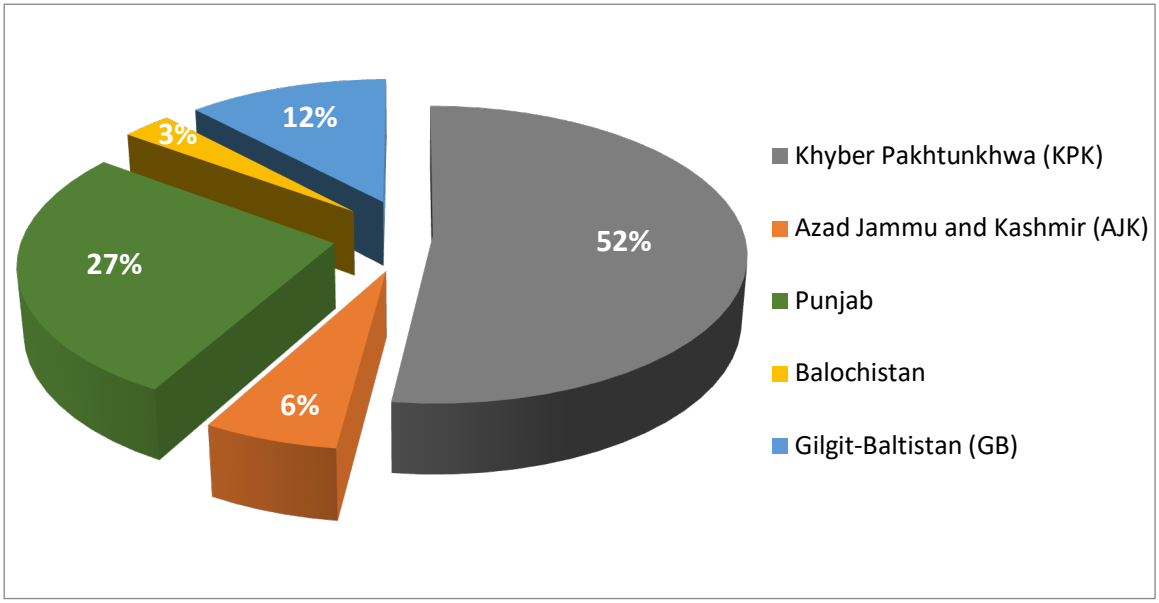


Figure 2. Area wise distribution of ethnobotanical studies of *Cannabis* from Pakistan

Aziz *et al.* (2016) validated ethnobotanical uses of *C. sativa* against asthma, urinary tract diseases, insomnia and depression because of its analgesic and sedative effects from Ladha South Waziristan. According to Aziz *et al.* (2016) the plant gives cooling effect when used in summer. A study from Hazar Nao Malakand unveiled the ethnobotanical uses of shoots and leaves of *C. sativa* as sedative and analgesic. Uses of *C. sativa* leaves against hallucination and to heal wound were also validated (Amin *et al.* 2018).

Hussain *et al.* (2018) described the uses of extracts from stem and leaves of *C. Sativa* as analgesic, sedative, narcotic and aphrodisiac from Kurram. Khan *et al.* (2018) found that the locals of Mandan, Bannu uses leaves of wild *C. sativa* as expectorant and used to treat fever and pain.

A study from Manoor valley (Rahman *et al.* 2019) documented the uses of *C. sativa* leaves against warmness and insomnia. A study from Kiwai, Kaghan validated the uses of leaves, stem and flowers of *C. sativa* plant against stomach problems and taken as analgesic to control problems during pregnancy (Akhtar *et al.* 2021). In the Swabi and Hazara, leaves of *C. sativa* were used to treat cancer, anthrax, HIV/AIDS, muscle atrophy blood poisoning, malaria, weight loss and dysentery. Its uses as pain killer and antidandruff were also reported (Hussain *et al.* 2022).

In the Punjab province, Hussain *et al.* (2010) found that the whole *Cannabis* plant was used to minimize intoxication, general body inflammation and appetite loss in the Jalalpur Jattan region of Gujrat. From the Pind dadan khan Jehlum, Iqbal *et al.* (2011) reported the uses of whole *C. sativa* plant as a tonic, intoxicant, stomachic, narcotic and sedative.

Ajaib *et al.* (2014) reported the ethnobotanical data of *Cannabis* from the Kharain and found that the flowers and leaves of *C. sativa* were used as sedative and a suitable remedy against whooping cough, piles, constipation and stomach ailments. It is also taken against persistent headache and menstrual cycle by the locals of Kharian. Moreover, it is used as narcotic in the form of 'hashish' obtained by rubbing the leaves.

From the Miani Sahib Lahore, Shah *et al.* (2016) reported folk traditional uses of *C. sativa* against headache, asthma, leucorrhea, cataract, palpitation and nose bleeding. Umair *et al.* (2017) documented the traditional uses of *C. sativa* leaves, seeds and whole plant from Hafizabad as a remedy against asthma, diarrhea, constipation, and snake bite. Its uses as sedative, diuretic, lice killer, laxative and intoxicant were also acknowledged. Uses of *C. sativa* against insomnia were also reported from Namal region by Shah *et al.* (2019). They validated the uses of *C. sativa* against abdominal pain, hypertension and toothache In Gujrat, various health problems including muscle pain, burns, flu, cough, asthma, loss of appetite, headache, bronchitis and inflammations in body, are cured with *C. sativa* (Ali *et al.* 2020).

In the Balochistan province, Bibi *et al.* (2014) unveiled the folk traditional aspects of *C. sativa* from the Mastung and documented the folk uses as a remedy to make the babies sleep at night.

Table 1. Folk traditional uses of *Cannabis* reported from different regions of Pakistan

Region	Local name	Part used	Folk medicinal uses	Reference
Booni Chitral, KPK	Bong	Leaves, seeds and floral bud	Used against jaundice, bronchitis, malaria	Ahmad *et al.* (2006)
Ranyal hills, Shangla, KPK	Bhung	ND	Used as sedative, narcotic and fiber	Ibrar *et al.* (2007)
Jalalpur Jattan Gujrat, Punjab	Bhang, Indian Hemp	Whole plant	Used to reduce intoxication, general body inflammation and appetite loss	Hussain *et al.* (2010)
Kabal Swat, KPK	Bhang	Leaves	Leaves juice is used against malaria and taken as pain killer remedy and flatulence. Its leaves juice has significant effects against male impotency and colic pain. Used to make hashish (chars) from female plant	Ahmad *et al.* (2011)
Malaknd Pass Hills KPK	Bhang	Shoot	Used as hypnotic, narcotic and sedative	Barkatullah & Ibrar (2011)
Battagram, KPK	Bhang	Leaves	Used as sedative, anodyne and narcotic	Haq *et al.* (2011)
Pind dadan khan, Jehlum, Punjab	Bhang, charas, ganja	Whole plant	Used as tonic, intoxicant, stomachic, narcotic and sedative	Iqbal *et al.* (2011)
Kotli AJK	Bhang	Leaves, stem and roots	Used as medicine and marketing purpose	Ahmad *et al.* (2012)
Gilgit GB	Bhang/ Thunchi	Whole plant	From the resin of leaves, stem, flowers and fruits, a strong narcotic is obtained. Used as sedative, narcotic, intoxicant and analgesic. Fiber from *C. sativa* is also obtained by the locals of the studied region	Awan *et al.* (2013)
Hunza GB	Thoonch	Seeds	Roasted seeds are expectorant and effective for throat sour and irritation	Khan *et al.* (2013)
Kharian Gujrat, Punjab	Bhang	Whole plant	Leaves and flowers are used as sedative and narcotic. Hashish (chars) is made from leaves. Leaves are used for the treatment of piles, whooping cough stomach disorders and constipation. Used against persistent headache and to control menstrual cycle	Ajaib *et al.* (2014)
Mastung, Balochistan	Bhang	Leaves and seeds	Used as narcotic. Given to the babies as sleeping remedy at night	Bibi *et al.* (2014)
Dir lower KPK	Bhang	Leaves and stem	Used in cigarette, also as diuretic and stimulants; leaves are taken with sugar water and used for treatment of inflammation of stomach, indigestion and liver diseases.	Shuaib *et al.* (2014)

			Used to make tandai to keep the body cool during summer	
Ghizer Gilgit-Baltistan	Bung	Whole plant	Plant powder is used to treat bronchitis cough and chest related ailments	Jabeen *et al.* (2015)
Mohmand Agency, KPK	ND	Whole plant	The dried plant is used for burning purposes	Ali *et al.* (2016)
Ladha South Waziristan, KPK	Bhangay	Flowering stems of female plants leaves	Used to treat asthma, urinary tract diseases, depression and insomnia due to its analgesic and sedative effects	Aziz *et al.* (2016)
Darazinda F.R. D.I. Khan, KPK	ND	ND	Some medicinal uses	Samreen *et al.* (2016)
Miani Sahib Graveyard Lahore, Punjab	Bhung	Whole plant	Used against cataract, asthma, headache, nose bleeding, leucorrhoea and palpitation	Shah *et al.* (2016)
Hafizabad Punjab	Bhang	Leaves, seeds, whole plant	Used against diarrhea, constipation, asthma and snake bite. Used as sedative, intoxicant, lice killer, diuretic, laxative	Umair *et al.* (2017)
Hazar Nao Malakand, KPK	Bhang	Shoots and leaves	Used as sedative, narcotic and analgesic Leaves against hallucination, and to animal wound healing	Amin *et al.* (2018)
Kurram agency, KPK	ND	Leaves and stem	Extract of leaves and branches are narcotic, analgesic, sedative and aphrodisiac	Hussain *et al.* (2018)
Mandan, Bannu, KPK	Bhang	Leaves	It is used to treat fever and pain and used as as expectorant	Khan *et al.* (2018)
Charsadd, KPK	Bhang	Leaves and flowering buds	Used as laxative, sedative, stimulant, diuretic and narcotics	Khan *et al.* (2018)
Haramosh GB	Thunchi	Leaves	Seeds are used to increase milk productivity in animals and humans. Leaves are taken against measles, chicken pox and stomach problems	Abbas *et al.* (2019)
Manoor valley KPK	Bhang	Leaves	Used against insomnia	Rahman *et al.* (2019)
Namal valley salt range, Punjab	Bhang	Leaves	Used to treat insomnia, hypertension, toothache and abdominal pain	Shah *et al.* (2019)
Gujrat Punjab	Bhaang	Whole plant	Used against loss of appetite, intoxication, asthma, inflammation of body, headache, cough, flu, bronchitis and pains. Burn and muscular pain are also treated with this plant	Ali *et al.* (2020)

Harighal AJK	Bhang	Leaves and flowers	Used as stringent that bowel stomachic and used as tonic and narcotic. Also used against leprosy	Amjad *et al.* (2020)
Gujrat Punjab	ND	Leaves and seeds	Used against diarrhea, constipation. Externally used against snake bite.	Rehman *et al.* (2020)
Gokand Buner KPK	Bang	Leaves	Used as sedative, narcotic and pain killer. Used to treat ulcer	Sulaiman *et al.* (2020)
Kiwai, Kaghan KPK	Bhang	Leaves, stem and flowers	Used to overcome pregnancy problems. Used to cure stomach problems and as analgesic	Akhtar *et al.* (2021)
Head Maralla Punjab	Bhang, Indian hemp	Whole plant	The plant is used for stomach and as narcotic	Iqbal *et al.* (2021)
Swabi and Hazara KPK	Bhang	Leaves	Narcotic drug used against blood poisoning, anthrax, malaria, cancer, dysentery, HIV/AIDS, muscle atrophy and dramatic weight loss. Used as antidandruff and pain killer	Hussain *et al.* (2022)

GB = Gilgit-Baltistan, KPK = Khyber Pakhtunkhwa, ND = Not defined

In the Gilgit-Baltistan region, it has been validated that a strong narcotic from the resin of leaves, stem, flowers and fruits of *C. sativa* plant is obtained and used as narcotic, sedative, intoxicant and analgesic by the locals (Awan *et al.* 2013). Khan *et al.* (2013) evaluated the folk traditional uses of *Cannabis* from Hunza and found that the roasted seeds of *C. sativa* plant are expectorant and most effective remedy for throat sour and irritations. Jabeen *et al.* (2015) reported uses of *C. sativa* whole plant as a remedy against cough, bronchitis and chest problems from Ghizer. From the distant Haramosh, Abbas *et al.* (2019) authenticated the uses of *C. sativa* leaves as a remedy against stomach problems, chicken pox and measles.

In the Azad Jammu and Kashmir region, Amjad *et al.* (2020) studied ethnobotany of Harighal, where the uses of *C. sativa* against leprosy and as stringent and tonic were defined. Sulaiman *et al.* (2020) validated folk uses of *C. sativa* against ulcer from Gokand Buner KPK. Based on the collected data of this review (Table 1), it was found that almost all parts of the *Cannabis* plant specifically the leaves are used as widely as folk traditional medicine in Pakistan. The preparation of the remedy includes decoction, and its mode of administration involves oral intake or applied externally applied.

Phytochemistry of *Cannabis* from Pakistan

Cannabis chemical composition is very complex because of wide variety of chemical components and their interfaces with each other. These compounds belong to different classes of chemicals like steroids, flavonoids, mono and sesquiterpenes, hydrocarbons, amino acids and nitrogenous compounds (ElSohly & Slade 2005). The natural compounds recognized in *Cannabis* plant are unceasingly increasing; for example, in 1980, 423 compounds were reported (Turner *et al.* 1980), in 1995, 483 were recognized (Ross & ElSohly 1980-1994) and in 2008, more than 525 compounds were acknowledged (Choi *et al.* 2004; Radwan *et al.* 2008; ElSohly & Slade 2005; Ahmed *et al.* 2008). In *C. sativa,* cannabinoids together with their transformation products and analogues are the characteristic carbon 21 groups of compounds present (ElSohly & Slade 2005). Among these compounds, cannabigerol (CBG) was the first compound to be isolated from marijuana resin (Gaoni & Mechoulam 1964). Moreover, in 1940, cannabidiol (CBD) group of compounds was discovered with negative optical rotation and trans-absolute configuration. Correspondingly, cannabicyclol (CBL) are the compounds which were first described to have structural similarity with compounds of trans-tetrahydrocannabinol (-THC) type (Korte & Sieper 1964) but later were recognized as separate class of compounds on the basis of X-ray analysis and nuclear magnetic resonance (Begley *et al.* 1970; Kane 1971). A few miscellaneous types of cannabinoids including fatty acids, terpenoids, carbohydrates, flavonoids, hydrocarbons, aldehydes, simple alcohols, ketones, esters, acids and lactones were also acknowledged (Choudhary *et al.* 2013).

The phytochemistry of *Cannabis* (Table 2) has been evaluated by a number of researchers from Pakistan (Anwar *et al.* 2006; Khan *et al.* 2011; Isahq *et al.* 2015; Tayyab & Shahwar 2015; Naz *et al.* 2016; Ahmad *et al.* 2018; Ullah *et al.* 2018; Waris *et al.* 2018; Javaid *et al.* 2021; Nawaz *et al.* 2021) and reported more than 40 compounds (Fig. 3) suggesting that the *Cannabis* is rich in phytochemicals with significant biological activities.

Anwar *et al.* (2006) studied old, pressed content of *C. sativa* (hemp) seeds oil from Lahore, Jhelum and Swat which represents three different agro-ecological zones. They found maximum linoleic acid levels (56.50-60.50%) followed by α-linolenic (16.85 to 20.00 %), oleic (10.17 to 14.03 %), palmitic (5.75 to 8.27 %), stearic (2.19 to 2.79 %) and γ-linolenic acids (0.63 to 1.65 %). In the non-degummed oils, tocopherols α were found to be 54.02 to 60.40, tocopherols γ were 600.00 to745.00 and tocopherols δ were 35.00 to 45.60. After degumming, tocopherols α were reduced to 29.90 to 50.00, tocopherols γ were reduced to 590.00 to 640.00, and tocopherols δ was reduced to 30.40.

Haq *et al.* (2011) studied the ethanolic leaves extracts of *C. sativa* from Battagram and reported phytocmicals including cannabisativine, anhydrocannabisativine, N-(p-hydroxy-β-phenylethyl)-phdroxy-(trans)-cinnamide and cannabinoids. Khan *et al.* (2011) evaluated phytochemistry of twenty different medicinal plants, including *C. sativa* from Peshawar KPK where anthraquinones, reducing sugar, terpenoids, saponins, flavonoids, alkaloids, cardiac glycosides and tannins were reported in the ethanol extract of whole *C. sativa* plant.

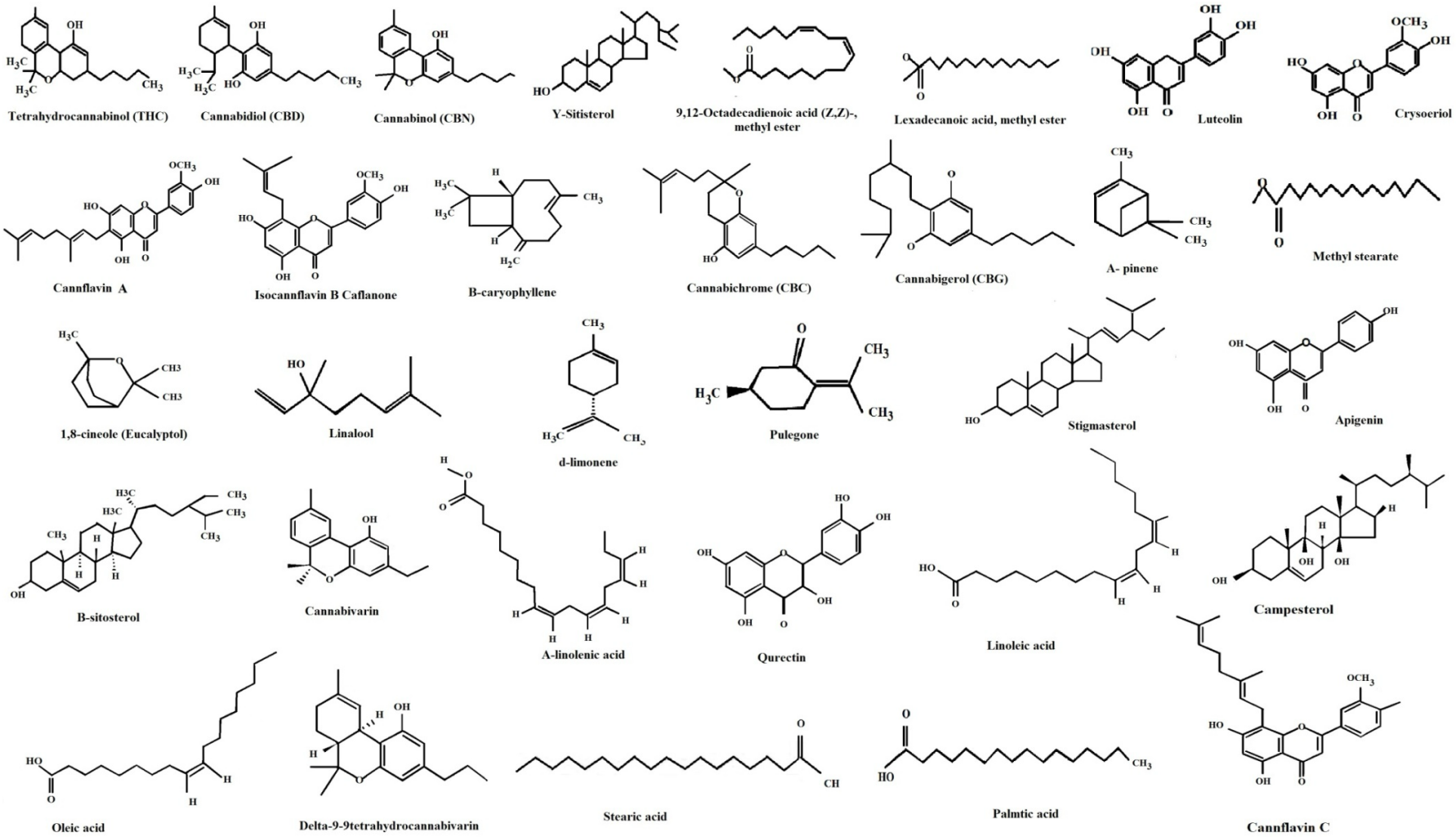
Isahq *et al.* (2015) investigated the phytochemical profile of *n*-hexane extracts of *C. indica* leaves, stems, and seeds from Peshawar KPK using GC-MS with emphasis of antimicrobial activities. They acknowledged the presence of saponins, alkaloids, tannins, sterols, flavonoids and terpenoids in *C. indica* with remarkable antimicrobial potential. A high percentage of some of the biologically active cannabinoids like cannabidiol (resorcinol, 2-p-mentha-1,8-dien-4-yl-5-pentyl) (50.077 %) and delta-9-tetrahydrocannabinol (25.040 %) were reported from *Cannabis* resin.

Table 2. Phytochemistry of *Cannabis* reported from different regions of Pakistan

***Cannabis* spp.**	**Region**	**Part used**	**Extraction solvent /extraction method**	**Phytochemicals/derivatives**	**Detection method**	**Reference**
C. sativa	Lahore, Jhelum Punjab and Swat KPK	Seeds	Oil extract/cold pressing extraction	α-linolenic, linoleic acid, oleic, stearic, palmitic, α, γ, and δ tocopherols, γ-linolenic acids,	HPLC	Anwar *et al.* (2006)
	Peshawar KPK	Whole plant	Ethanol/soaking extraction	Anthraquinones, reducing sugars, terpenoids, saponins, flavonoids, cardiac glycosides and alkaloids	Classical method	Khan *et al.* (2011)
C. indica	Peshawar KPK	Leaves, stems and seeds	*n*-hexane	Cannabivarin, cannabichromene, delta-9-tetrahydrocannabinovarin, cannabidiol (resorcinol, 2-p-mentha-1,8-dien-4-yl-5-pentyl), 6,6,9-trimethyl-3-pentyl-6H-dibenzo(b,d)pyran-ol, cannabigerol and delta-9-tetrahydrocannabinol	GC-MS	1sahq *et al.* (2015)
C. sativa	Kashmir AJK, Bhakkar, Gujranwala and Chaman Punjab	Whole plant	*n*-hexane	Delta 9-tetrahydrocannabivarin, 4-azapyrene, cannabidiol, 9-anthracenecarbonitrile, phytol, acenaphthol, cannabinol and delta-9-tetrahydrocannabinol		Tayyab & Shahwar (2015)
	ND	ND	Methanol and its 10 fractions	Ferulic acid, trans-4-hydroxy-3 methoxy cinnamic acid, p-coumaric acid, flavonoids, lycopene, flavonols, beta-carotenes and ascorbic acid	HPLC	Naz *et al.* (2016)
	Kacha, Layyah Punjab	Whole plant and seeds	Methanol	p-coumaric acid, quercetin, m-coumaric acid, cinnamic acid, gallic acid, caffeic acid, ferulic acid, kampferol and benzoic acid		Ahmad *et al.* (2018)
	Bajaur agency KPK	Leaves	Methanol, ethanol and chloroform/soaking extraction	Flavonoids, alkaloids, phlobatannins, tannins, carbohydrates, saponin, phenols, cardiac glycosides, glycosides, proteins and terpenoids	Classical method	Ullah *et al.* (2018)
	Peshawar KPK	Whole plant	Aqueous/boiling extraction	Flavonoids, tannins, alkaloids, glycosides, triterpenoids, saponins and steroids		Waris *et al.* (2018)

C. sativa* ssp *indica	Bahawalnagar Punjab	Leaves and roots	Methanol, chloroform, ethanol, ethyle acetate, hexane, acetone, distilled water/cold extraction	Alkaloids, phenols, catecholic acid, saponins, glycosides, flavonoids, steroids and flavones		Ahmed *et al.* (2019)
C. sativa	Lahore Punjab	Roots/ soaking	Methanol	9,12-octadecadienoic acid (Z,Z)-, methyl ester, γ-sistosterol, methyl stearate, hexadecanoic acid methyl ester, stigmasterol, phenol, campesterol, 11-octadecenoic acid ester, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl, 2-methoxy-4-vinylphenol, tetracosanoic acid methyl ester, and dronabinol	GC-MS	Javaid *et al.* (2021)
C. sativa	Gujranwala and Narowal Punjab	Seeds	Hexane/ soxhlet extraction	α-tocopherol, γ-tocopherol and phenols	UV- Spectro	Nawaz *et al.* (2021)

GC-MS; Gas chromatography-Mass spectroscopy, HPLC; High performance liquid chromatography, KPK; Khyber Pakhtunkhwa, ND; Not defined, UV-Spectro; Ultraviolet visible spectrophotometer


Tetrahydrocannabinol (THC)
Cannabidiol (CBD)
Cannabinol (CBN)
Y-Sitisterol
9,12-Octadecadienoic acid (Z,Z)-, methyl ester
Lexadecanoic acid, methyl ester
Luteolin
Crysoeriol
Cannflavin A
Isocannflavin B Caflanone
B-caryophyllene
Cannabichrome (CBC)
Cannabigerol (CBG)
A- pinene
Methyl stearate
1,8-cineole (Eucalyptol)
Linalool
d-limonene
Pulegone
Stigmasterol
Apigenin
B-sitosterol
Cannabivarin
A-linolenic acid
Qurectin
Linoleic acid
Campesterol
Oleic acid
Delta-9-9tetrahydrocannabivarin
Stearic acid
Palmtic acid
Cannflavin C

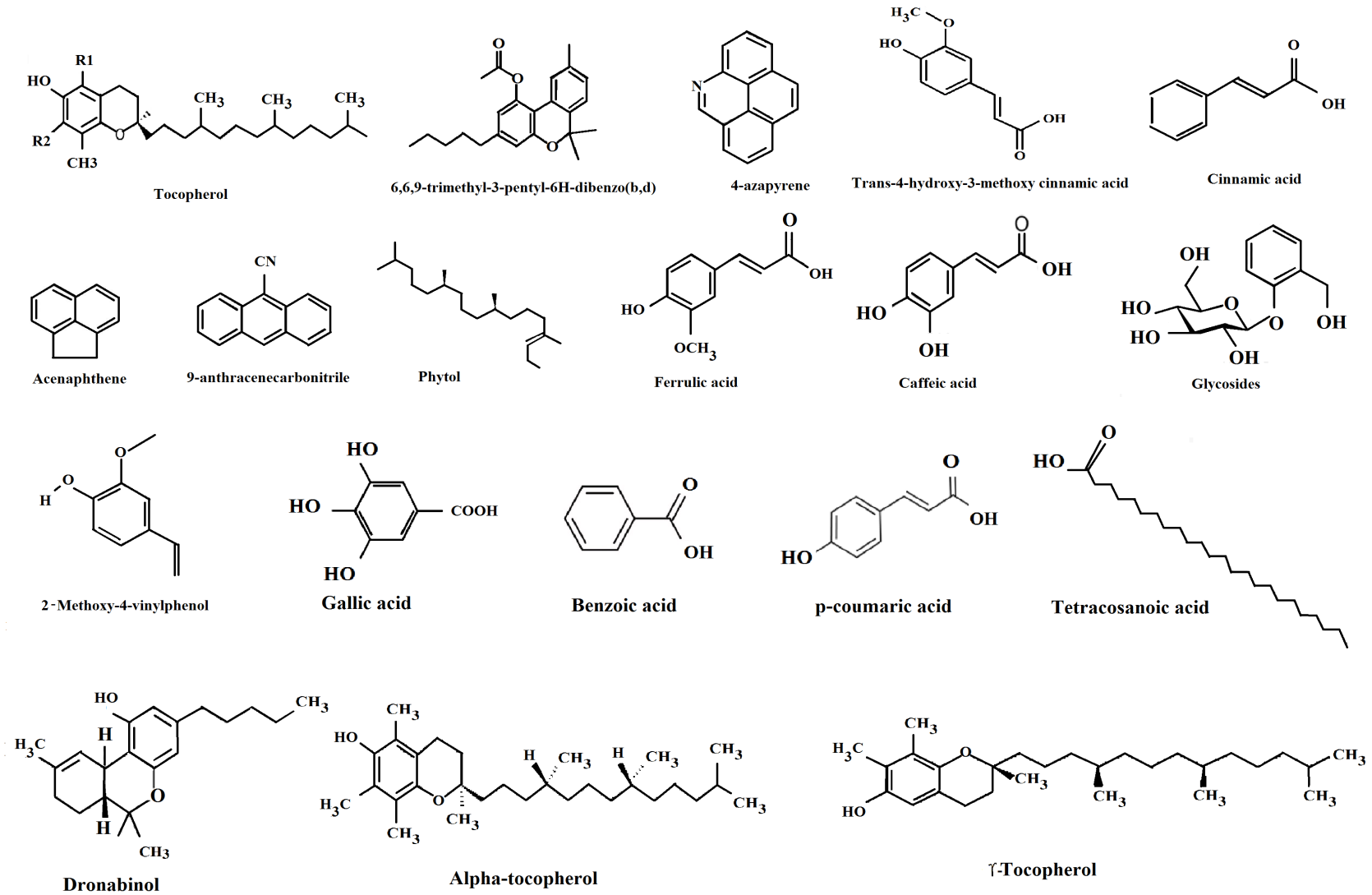


Figure 3. Important group of phytochemicals reported in *Cannabis* from Pakistan (Anwar et al. 2006; Isahq et al. 2015; Ahmed et al., 2018; Javaid et al. 2021).

In a study, *Cannabis* samples obtained from Bhakkar, Kashmir, Gujranwala and Chaman were investigated using GC-MS technique where the *n*-hexane extracts of *C. sativa* were rich in main psychoactive constituents. D-9 tetrahydro cannabinol was the most plentiful compound in the tested samples. Chemical compounds within the samples obtained from two regions (Kashmir & Bhakkar) possess same and the compounds reported in all *C. sativa* samples recognized were delta 9-tetrahydrocannabivarin, 4-azapyrene, cannabidiol, cannabinol, phytol, 9-anthracenecarbonitrile and acenaphthol (Tayyab & Shahwar 2015).

Naz *et al*. (2015) examined the antioxidant activities and phenolics analysis of crude methanolic extract fractions of *C. sativa*. Ten fractions have been collected from partition fractionation of crude methanolic extract. Phenolics were the major antioxidant component detected in the methanol extracts (314.3 mg/g), followed by flavonoids (225 mg/g), flavonols (210 mg/g), ascorbic acid (125 mg/g), beta-carotenes (0.45mg/g) and lycopene (0.09 mg/g). HPLC analysis showed three major phenolics, p-coumaric acid (11.44-6.91 mg/l), ferulic acid (7.83-4.10 mg/l) and trans-4-hydroxy-3 methoxy cinnamic acid (7.13-3.15 mg/l) in methanol, ethanol and2-butanol fractions.

Ahmad *et al*. (2018) characterized the whole *C. sativa* plant methanol extract and seed for non-cannabinoid compounds identification with HPLC technique. The presence of p-coumaric acid, gallic acid, quercetin, caffeic acid, m-coumaric acid, ferulic acid, cinnamic acid, kampferol and benzoic acid were confirmed. Additionally, high concentrations of quercetin in whole plant were observed, whereas absence of m-coumaric acid and high gallic acid was acknowledged from the *Cannabis* seed. Plant samples had higher concentration of cinnamic acid as compared to the seed samples. Low concentrations of benzoic acid, caffeic acid and ferulic acid were documented in *Cannabis* plant and seed samples (Ahmed *et al*. 2018).

Ullah *et al*. (2018) collected *C. sativa* from Bajaur agency and investigated the qualitative and quantitative phytochemistry with antibacterial activity of ethanolic, chloroform and methanolic leaf extracts. It has been shown that *C. sativa* was rich in phlobatannins, alkaloids, tannins, carbohydrates, flavonoids, phenols, cardiac glycosides, saponin, proteins, terpenoids and glycosides. Higher content of phenol (0.68 mg/g) was found in the *C. sativa* methanolic extract with potential antibacterial activity.

Waris *et al*. (2018) unveiled the phytochemistry of aqueous *C. sativa* (whole plant) extracts from Peshawar and confirmed the presence of tannins, glycosides, alkaloids, saponins and flavonoids in the *C. sativa* extracts.

In a study from Bahawalnagar, ethanol, methanol, ethyle acetate, acetone, chloroform, aqueous and hexane extracts of roots and leaves of *C. sativa* ssp. *indica* (hemp) were reported with phytochemicals like catecholic acid, phenols, saponins, alkaloids, glycosides, steroids, flavonoids, flavones The methanol was designated with maximum content of phenols from *C. sativa* (37.69 mg gallic acid equivalent GAE/g) and flavonoids in the leaves was 59.03 mg quercetin equivalent QE/g. A higher DPPH inhibition has been recorded for acetone (55.57%), hexane (45.98%) and distilled water (35.5%) from roots and leaves of *C. sativa* (Amed *et al*. 2019). In another inquiry, phytochemical profile of methanol extract of roots of *C. sativa* collected from Lahore was assessed through GC-MS technique and more than fourteen compounds were identified (Javaid *et al*. 2021). The most abundant compounds were γ-sitosterol, 9,12-octadecadienoic acid (Z,Z)-, an hexadecanoic acid methyl ester and with varying concentrations. Other identified compounds were β-amyrin, methyl stearate, stigmasterol, campesterol, 2,2' methylenebis[6-(1,1-dimethylethyl)-4-methyl, phenol, squalene, 11-octadecenoic acid, methyl ester, 2- methoxy-4-dronabinol, methyl ester, eicosanoic acid, tetracosanoic acid, methyl ester, vinylphenol (Javaid *et al*. 2021). Moreover, the identified compounds were designated to have anticancer, antidiabetic, analgesic, anti-inflammatory, antimicrobial, antioxidant and antitumor activities. Nawaz *et al*. (2021) characterized the hexane extract of *C. sativa seeds* from Gujranwala for the identified compounds with UV-Spectroscopy technique and confirmed the presence phenols, α-tocopherol and γ-tocopherol. It was validated that future prospects resides in the idea that *Cannabis* is a valuable crop and could be explored further for commercial and edible purposes. Overall, the Phyto-chemistry data reviewed here showed that more than 70 different compounds were identified reported in *Cannabis* species from Pakistan with potential biological activities.

Biological activities of *Cannabis* from Pakistan

Antimicrobial activity

For numerous deadly diseases associated with microbes, the main accountable bacterial pathogens are *Staphylococcus*, *Salmonella, Bacillus* and *Pseudomonas* (Ahameethunisa & Hoper 2010). Because of subcutaneous, superficial and deep inside complications, pathogenic fungi are also liable for many diseases and are increasing

globally (Shaik 2011). The tendency of drug resistance by microorganisms is a great risk for the human health. To cope with this issue, medicines herbal sources are considered worldwide as a substitute of synthetic antimicrobial drugs. For treating many diseases plants used traditionally. In the human medical exercise, a lot of plants from different genera are used traditionally against bacterial and fungal pathogens and there has been no drug resistance sign seen till now. One of the medicinally significant plants *C. sativa* has shown promising results against certain kind of pathogenic microbes due to the presence of vital chemical elements. Presently, the medicinal uses of *C. sativa* are wide-opening in some parts of the world with the potential to treat diseases from headaches to nervous disorders and cancer etc. (Esra *et al*. 2012).

In Pakistan, the two *C. sativa* and *C. indica* were extensively studied by many researchers (Wasim *et al*. 1995; Malik *et al*. 2011; Yasmeen *et al*. 2012; Hazrat *et al*. 2013; Iqbal *et al*. 2014; Naveed *et al*. 2014; Isahq et al. 2015; Rashid *et al*. 2016; Anjum *et al*. 2018; Ullah *et al*. 2018; Khan and Javaid 2020; Rida *et al*. 2020; Zaka *et al*. 2021) and reported the antimicrobial activities of their extracts and validated *Cannabis* as potential natural antimicrobial agent (Table 3).

An *In vitro* antimicrobial activity of ethanolic, aqueous and petroleum ether extracts of *C. sativa* leaves was assessed from Lahore and Jehlum (Wasim *et al*. 1995). A strong antimicrobial activity of petroleum ether, ethanol, and the acidic fractions against gram-positive and gram-negative bacteria and fungal strains were acknowledged with no antimicrobial activity in the aqueous extract.

Malik *et al*. (2011) collected *C. sativa* from Islamabad, Punjab, Sarghodha Division, Kalar Kahar, and Hazara and reported moderate to good activity of aqueous, ethanolic and *n*-hexane extracts against gram positive and gram-negative bacterial strains specifically the yeast species and enteric pathogens.

In another study from Lahore, alcoholic and aqueous extracts of fresh leaves, dry seeds and rhizomes of *C. sativa* and four commercial products were *in vitro* screened for antibacterial activity against *Proteus mirabilis* using the serial dilution procedure (Yasmeen *et al*. 2012). The outcomes validated strong antibacterial activity against the tested *P. mirabilis* strain.
Hazrat *et al*. (2013) checked the efficacy of methanol extract of *C. sativa* for antimicrobial activity from Dir Kohistan against *E. coli, S. aureus*, *S. typhi, P. aeruginosa, B. cereus* and *B. subtilis*. The obtained crude extract and successive fractions of *C. sativa* demonstrated moderate to outstanding activities at 200 mg/ml concentration with mean inhibition zones of 23.3 mm for *E. coli*, 13.66 mm for *B. subtilis*, 11.33 mm for *B. cereu,* 13.33 mm for *S. aureus,* 14.33 mm for *P. aeruginosa* and 24.0 mm for *S. typhi*. The methanol fraction overall exhibited maximum antibacterial activity in *C. sativa.*

Iqbal *et al*. (2014) studied antifungal potential of aqueous leaves extracts of *C. sativa* from Islamabad against *Macrophomina phaseolina* which is the most harmful phyto-pathogenic fungal species. The tested extracts of plant displayed growth of *M. phaseolina* considerably to fluctuating levels.

Naveed *et al*. (2014) studied the *C. sativa* aqueous and ethanol leaf extracts activity from Mansehra KPK against *E. coli, S. aureus, P. aeruginosa*, *S. typhi, E. faecalis*, and *Klebsiella* with well diffusion method. They displayed highest zone of inhibition in the ethanol extract against the tested bacterial strains. *C. Sativa* leaves exerted better antibacterial activity against with zone of inhibition of 24.1 mm for *S. aureus*, 10.3 mm for *P. aeruginosa*, 22.2 mm for *E. coli* and 18.1 mm for *E. faecalis* and was not effective against *Klebsiella* and *S. typhi.* Naveed *et al*. (2014) proposed that the minimum inhibitory effect of *C. sativa* leaf extract might be due to the presence of chemical compounds present in *C. sativa*.

In a study from Lahore, Rashid *et al*. (2015) observed a significant elevated activity against tested microbes in the *C. sativa* ethanol leaf extracts with greater zone of inhibitions against *S. aureus, S. aureus, K. pneumonia* and *E. coli*. It has been validated that *C. sativa* possess wide-ranging scale of antimicrobial activity and contains natural antioxidants with substantial pharmaceutical reputation.

Table 3. Antimicrobial activity of *Cannabis* reported from different regions of Pakistan

***Cannabis* spp.**	**Region**	**Part used**	**Extract used**	**Extract conc.**	**Microorganisms tested**	**Results**	**Reference**
C. sativa	Lahore and Jehlam Punjab	Leaves	Aqueous, ethanolic and petroleum ether extracts	1, 5 and 10 mg	*B. pumilus, B. subtilus, S. aureus, Proteus vlugaris, M. flavus, C. albicans, A. niger* and *B. bronchioseptica,*	+	Wasim *et al.* (1995)
C. sativa	Islamabad, Sarghodha Kalar Kahar Punjab, and Hazara KPK	ND	Aqueous, ethanolic and *n*-hexane	ND	*E. coli, B. subtilis, M. pyogens, K. pneumoniae, S. typhi, S. aureus, S. dysentriae, S. epidermidis, V. cholera* and *S. cerevisiae*	–	Malik *et al.* (2011)
C. sativa	Lahore, Punjab	Fresh leaves, rhizome and dry seeds	Aqueous and alcoholic	ND	*Proteus mirabilis*	+	Yasmeen *et al.* (2012)
C. sativa	Dir Kohistan KPK	Leaves	Methanol	100mg	*E. coli, S. aureus, P. aeruginosa, B. subtilis, B. cereus* and *S. typhi,*	+	Hazrat *et al.* (2013)
C. sativa	Islamabad	Leaves	Aqueous	ND	*Macrophomina phaseolina*	+	Iqbal *et al.* (2014)
C. sativa	Mansehra KPK	Leaves	Aqueous and ethanol	100µl	*E. coli, S. aureus* and *P. aeruginosa*	+	Naveed *et al.* (2014)
C. indica	Peshawar KPK	Leaves, stems and seeds	Chloroform, methanol, *n*-butanol	10 µg	*B. cereus, S. aureus, P. mirabilis, K. pneumoniae, A. parasiticus, A. niger* and *A. oryzae*	+	Isahq *et al.* (2015)
C. sativa	Lahore Punjab	Leaves and stems	Ethanol and methanol	ND	*P. aeruginosa, K. pneumonia, S. aureus* and *E. coli*	+	Rashid *et al.* (2016)
C. sativa	Bhimber Azad Jammu and Kashmir	Leaves	Acetone, chloroform, 60% ethanol and distilled water	20µl	*E. coli, S. aureus, P. aeruginosa, Fusarium spp.* and *A. niger*	+	Anjum *et al.* (2018)
C. sativa	Bajaur agency KPK	Leaves	Chloroform, methanol and ethanol	75 µl	*S. aureus, S. flexneri, P. aeruginosa E. coli,* and *S. typhi*	+	Ullah *et al.* (2018)
C. sativa	Lahore Punjab	Leaves	Methanol fractions (aqueous, *n*-Butanol, *n*-hexane, Ethyl acetate)	1.562 to 200 mg mL-1	*A. flavipes*	+	Khan & Javaid (2020)
C. sativa	Gilgit-Baltistan	Whole plant	Ethyl acetate	1.56 mg/ml	*Pseudomonas* and *Bacillus* strains	+	Rida *et al.* (2020)
C. sativa	Islamabad	Leaves	Callus extract	3-5 mg	*S. epidermidis, B. subtilis, K. pneumonia, A. Fumigatus, P. aeruginosa, F. solani, A. niger, Mucor* and *A. flavus*	+	Zaka *et al.* (2021)

KPK; Khyber Pakhtunkhwa, ND; Not defined

Chloroform, methanol, *n*-butanol extracts from stems, leaves and seeds extracts of *Cannabis* species like *C. indica* from Peshawar KPK exhibited elevated antibacterial activities when screened against *B. cereus*, *S. aureus*, *P. mirabilis* and *K. pneumoniae*. The extracts further displayed significant antifungal activities against *A. oryzae, A. parasiticus* and *A. niger*. It has been validated that the cannabidiol (resorcinol, 2-p-mentha-1,8-dien-4-yl-5-pentyl) (50.077%) and delta-9-tetrahydrocannabinol (25.040%) which are the biologically active cannabinoids present in high percentage in *C. indica* inhibiting the growth of pathogenic microbes (Isahq *et al*. 2015).

An *in vitro* study from Bhimber AJK, the potential antimicrobial action of leaf extracts of *C. sativa* with chloroform, acetone, ethanol and water tested against *S. aureus E. coli,* and *P. aeruginosa*, *Fusarium* spp. and *A. niger* species perceived positive results retarding the growth of tested microbes. Only the acetone and ethanol extracts of *C. sativa* uncovered maximum zone of inhibitions against the majority of tested strains. *C. sativa* chloroform extract exhibited lower activity against few microbial strains (Anjum *et al*. 2018).

Ullah *et al*. (2018) collected *C. sativa* from Bajaur agency, and the ethanol, methanol and chloroform extracts of its leaves confirmed antibacterial activity against *S. aureus*, *Shigella flexneri, P. aeruginosa, E. coli* and *S. typhi*. An elevated zone of inhibition (18.46 mm) in the chloroform extract of *C. sativa* against *S. typhi* was evident which was followed by ethanol extract (17.67 mm) against *S. aureus*.

Khan & Javaid (2020) assessed the antifungal effect of *C. sativa* leaf extract of against *Aspergillus flavipes* in Lahore. The partitioning of methanol leaf extract into five fractions was achieved in different organic solvents with different concentrations (1.562 to 200 mg mL-1). The fraction of *n*-Butanol pronounced maximum antifungal activity of 68-82% followed by chloroform 52-82% and *n*-hexane 42-82% against *A. flavipes*. A very moderate antifungal activity was perceived in the ethyl acetate extract with 47-76% biomass reduction and minimum antifungal activity of 38-73% was observed for the aqueous extract of the plant. Khan and Javaid (2020) further validated that the leaf extract of *C. sativa n*-butanol fraction was remarkably active against *A. flavipe.*

Extracts of *C. sativa* from the northeast Gilgit-Baltistan (Rida *et al*. 2020) was screened for antibacterial and antioxidant activities against *Bacillus* and *Pseudomonas* strains. Ethyl acetate extracts of *C. sativa* revealed the presence of different phytochemicals in all extracts. Maximum antibacterial activity against *Bacillus* (KC 881030) and *Pseudomonas* (KC 881031) strains was shown by *C. sativa* (14 mm). Its minimum inhibition concentration (MIC) was found to be 1.56 mg/ml against test strain of *Bacillus*.

Zaka *et al*. (2021) from Islamabad proposed a green synthesis of Ag and ZnO nanoparticles method with *C. sativa* callus extracts. The antibacterial activity of the synthesized nanoparticles from *C. sativa* evaluated against bacterial strains like *K. pneumonia, B. subtilis, P. aeruginosa* and *S. aureus,* and fungal strains like *A. flavus, Mucor, A. niger, F. solani* and *A. fumigatus* exhibited substantial antifungal and antibacterial activities with improved cytotoxic outcome. Overall, the data regarding the antimicrobial activity reviewed here showed that *Cannabis* extracts from 9 to 10 organic solvents were effective against ~19 bacterial and 8 fungal strains.

Antioxidant activity

Plants normally possess many phytochemicals in the extracts from different parts and their uses may be very productive for the development of new therapeutic methodologies for the treatment of many diseases. It could be only possible if the antimicrobial and antioxidant efficacies of the plant are known (Lewis & Elvin-Lewis 1995). Antioxidants are the compounds from plants origin that protect the cell by counteracting against reactive oxygen species (ROS) by hunting them (Liu 2003).

C. sativa plant has been reported to possess free radical scavenging actions in contradiction of a number of ROS species and the anti-aging ROS efficacies of this plant have also been projected for safe use of cosmetic (Abrams & Guzman 2015) but due to the insufficiency of data, it necessitates an extended investigation to acknowledge its safe uses in cosmetics (Stokes *et al*. 2000).

The antioxidant efficacy of extracts from *Cannabis* plant from Pakistan has been evaluated by many researchers (Nadeem *et al*. 2012; Naz *et al*. 2016; Ahmed *et al*. 2019; Ali *et al*. 2020; Rida *et al*. 2020; Hussain *et al*. 2021; Nawaz *et al*. 2021) and the details of antioxidant activity of *Cannabis* from Pakistan are given in Table 4.

Nadeem *et al*. (2012) assessed the *in vitro* radical scavenging and total antioxidant activities of leaves, stem and inflorescence organic extracts of both male and female *C. sativa* plants with total phenolic conents (TPC), DPPH free

radical scavenging, ABTS radical cation scavenging, TEAC, metal chelating activity and lipid peroxidation inhibition assays. Highest ABTS scavenging activity was evident for the fractions achieved in polar solvents. The TAEC values of *C. sativa* various extracts of different parts ranged from 144.46 to 1.47 mM trolox equivalents for chloroform fraction of FS and 1-butanol fraction of FI. The DPPH radical scavenging rate for the extracts displayed a wide range of antioxidant compounds. It was validated with the ammonium thiocyanate method, that all the *Cannabis* extracts possess a very noteworthy activity for the lipid peroxidation inhibition.

In a study from Bahawalnagar (Ahmed *et al.* 2019), ethanol, methanol, chloroform, ethyle acetate, acetone, aqueous and hexane extracts of roots and leaves of *C. sativa* sub sp indica (hemp) were documented to have possible antioxidant activity with DPPH method. A very remarkable DPPH inhibition (%) for *C. sativa* leaves was recorded for acetone and distilled water extracts (34.2±1.10 to 55.57±1.20%).

From Lahore Pakistan, Rashid *et al.* (2016) evaluated the methanol and ethanol leaves and stem extracts of *C. sativa* for antioxidant potential using DPPH method. A strong antioxidant activity of *C. sativa* extracts was validated with 29.4 ± 0.1% to 43.5 ± 0.1%.

Naz *et al.* (2016) evaluated the antioxidant potential and phenolic content analysis of ten fractions of crude methanolic extractof *C. sativa*. All the tested fractions showed appreciable antioxidant activities with inhibition of linoleic acid peroxidation and DPPH radical scavenging activity. Similarly methanol, ethanol, acetic acid and 2-butanol fractions showed higher reducing ability at 600 mu g/l concentration. Highest total antioxidant activity was exhibited by methanol fraction in both ferric thiocyanate (54.21%) and phosphomolybdenum (61.11%) assays.
Waris *et al.* (2018) performed antioxidant activity of *C. sativa* by DPPH scavenging assay from Peshawar KPK using ascorbic acid as a standard indicated that aqueous extracts of *C. sativa* showed sturdiest and approximately the same action with IC_{50} value of 353µg/ml.

In a split face topographic study, Ali *et al.* (2020) discovered the actions of dermocosmetic loaded medical *Cannabis* seed extract and evaluated the development on the skin surface in Asian volunteers. The antioxidant action of plant extract before and after adding of plant extract of a fabricated product was around 87% and 79%.

A study from northeastern Gilgit-Baltistan, free radical scavenging ability of ethyl acetate extracts of *C. sativa* plant exposed highest (68.9%) as compared to some other tested plant extracts (Rida *et al.*, 2020).

In another study from Gilgit-Baltistan (Hussain *et al.* 2021), the water extract of *C. sativa* leaves displayed significant antioxidant activity in contrast to the control ones. In the goat brain homogenate 522.6 and 659.97 µg/mL of inhibition against iron-induced lipid peroxidation was found while against nitroprusside induced lipid peroxidation of the brain, the inhibitions were 273.54 and 309.18 µg/mL. The extracts of *C. sativa* have favorable actions against DPPH radicals (96.04%) validating that *C. sativa* has potential to inhibit oxidants with therapeutic outcome against oxidative stress induced diseases.

Nawaz *et al.* (2021) proposed that *C. sativa* extract is rich in antioxidants and a significant phenolic content. It is a crop containing a high polyunsaturated acid content and antioxidants. From the seeds, hemp oil was extracted and 33% yield was acquired and it was compared for its TPC and antioxidants with the commercially available hemp oil using Folin-Ciocalteu reagent and DPPH assays. The presence of phenolic compounds in the tested extracts with strong antioxidant activity has been acknowledged.

Overall, the data regarding the antioxidant activity reviewed here (Table 4) showed that *Cannabis* extracts from 10 different organic solvents possess potential antioxidant activity and the DPPH method was widely employed to assess the antioxidant activity.

Anticancer activity
During the last few years, researchers have focused on the therapeutic vitality of cannabinoids from the *Cannabis* plant for the unveiling of positive consequences in patients with cancer (Guzmán 2018; Badowski 2017). Currently, two important *Cannabis* based medicines permitted by several regulatory drug agencies are Cesamet® (nabilone) and Marinol® (dronabinol) against cancer. The palliative efficacy of *Cannabis* based medicines also include the treatment of neuropathic pain induced by cancer. Other than these palliative significances, few cannabinoids from *Cannabis* possess potential anticancer actions (Guzmán 2003; Khan *et al.* 2016; Guindon & Hohmann 2011; Hinz &

Ramer 2019). There is plenty of data available that report the capability of cannabinoids in modulating various pathways of cellular signaling implicated in cancer cell migration, proliferation and/or death (Chakravarti *et al.* 2014). However, the central pathways are not completely described; literature has shown some vital evidence for the association of at least four mechanisms like apoptosis induction, transformed-cell growth direct inhibition through the mitogenic signal suppression, metastasis and inhibition of tumor angiogenesis (Velasco *et al.* 2016). Cannabinoids of phytogenic, synthetic and endogenous nature have revealed positive effects that modulate different proteins against cancer proliferation involved in the endo-cannabinoid system like the fatty acid amide hydrolase (FAAH), ionotropic receptor TRPV1 and G protein-coupled receptors CB1, CB2, and GRP55.

The anticancer effects of cannabinoids were first reported in 1975 by Munson *et al.* (1975). The administration of CBN, Δ9-THC and Δ8-THC from *Cannabis* plant both *in vitro* and *in vivo* demonstrated the inhibition of Lewis lung adenocarcinoma cell growth in mice. Since then, the antimetastatic, anti-angiogenic, pro-apoptotic and antiproliferative effects of cannabinoids proved their effectiveness in various types of cancer including glioma, lung, skin, lymphoma, thyroid, uterus, ovary, neuroblastoma, pancreas, prostate, colorectal, liver, and breast carcinoma with *in vitro* and *in vivo* models (Galve-Roperh *et al.* 2000; Maccrrone *et al.* 2000; Melk *et al.* 2000; Sánchez *et al.* 2001; Casanova *et al.* 2003; Sarfaraz *et al.* 2005; Blázquez *et al.* 2006; Caffarel *et al.* 2006; Carracedo *et al.* 2006; Carracedo *et al.* 2006; Cianchi *et al.* 2008; Preet *et al.* 2008; Caffarel *et al.* 2010; Appendino *et al.* 2011; Piñeiro *et al.* 2011; Vara *et al.* 2011; Aviello *et al.* 2012; Tariq *et al.* 2012; De Petrocellis *et al.* 2013; Borrelli *et al.* 2014; Romano *et al.* 2014; Milion *et al.* 2020 and references therein). During the literature search, no published data was available on the anticancer actions of *Cannabis* from Pakistan. The cancer effects of cannabinoids against more than 12 types of cancer from *Cannabis* reported worldwide are given in Table 5.

Legal status and regulation of *Cannabis* (hemp) in Pakistan

After the exclusion of *Cannabis* from a category of the most dangerous drugs of the world, many countries legalized *Cannabis* for medicinal and recreational purposes. The top 10 hemp producer countries in the world with area covered is given in Table 6. The United States of America, Canada, China, North Korea and France are on the top on the basis of land area used for hemp production (Zhao *et al.* 2021). Some other European countries are small producers of hemp. It has been estimated that currently, more than 30 countries globally are working on cultivation and production of hemp for medicinal purposes.

In Pakistan, wild or cultivated *Cannabis* have high concentration of psychoactive compounds like THC, therefore it is illegal for recreational use. However, considering the UN commission removal of *Cannabis* from a category, Pakistan's current government proclaimed legal uses of hemp in September 2020 that it would allow the industrial production of hemp containing high amounts of medicinal CBDs with no or less than 0.3% amount of psycho active THC. With the legalization of hemp, Pakistan will now fall in the hemp producing countries of the world. Moreover, the pronouncement of legalizing *Cannabis* came after the apprehension of the fact that there is a natural abundance of *Cannabis* in Pakistan. After acquiring the legal status in Pakistan with proper check and balance, hemp will be now utilized to make hemp-based products like hemp seeds, CBD oil, medical, food and industrial products. With the legalization of hemp, possibilities will be occurring in the international market to export non-psychoactive hemp and its derivatives. According to an estimate, Pakistan could boost up about $1 billion (€ 820 million) of revenue over the next three years by capturing a share in the booming market of hemp products containing CBDs.

Additionally, the industrial hemp market worldwide was worth around $25 billion and numerous countries were comforting laws directing products of *Cannabis* such as CBD oils. Besides the generation of revenue, the legalization of hemp in Pakistan could provide employment for more people who can cultivate and package hemp in the Government approved institutions for sale. It could also create some other job openings for individuals who can work in health promotion initiatives and educational sessions to encourage safer hemp uses.

Under the Control of Narcotics Substance Act (CNS) of 1997, it is illegal to produce, manufacture, extract, prepare, possess, offer for sale, sell, purchase or distribute *Cannabis* in Pakistan especially for recreational purpose.

Implementation of laws contrary to the uses of hard drugs is prioritized in Pakistan where the role of Anti-Narcotics Force (ANF) in this regard is vital in the monitoring of hemp after its legalization in Pakistan. The cultivation and production of hemp will be strictly regulated by the ANF that deals with trafficking problems and *drug* addiction inside the country.

Table 4 Antioxidant activity of *Cannabis* reported from different regions of Pakistan

***Cannabis* spp.**	**Region**	**Part Used**	**Extract Used**	**Method**	**Reference**
C. sativa	Shahdara, Lahore Punjab	Leaves, Stem and Inflorescence	chloroform, *n*-hexane, 1-butanol and ethyl acetate	ABTS, DPPH and TAEC	Nadeem *et al.* (2012)
C. sativa	Lahore Punjab	Leaves and stems	Ethanol and methanol	DPPH	Rashid *et al.* (2016)
C. sativa	ND	ND	Methanol and its 10 fractions	DPPH	Naz *et al.* (2016)
C. sativa	Peshawar KPK	Whole plant	Aqueous	DPPH	Waris *et al.* (2018)
C. sativa* ssp. *indica	Bahawalnagar Punjab	Leaves and roots	Ethanol, methanol, ethyl acetate, acetone, chloroform, distilled water and hexane	DPPH	Ahmed *et al.* (2019)
C. sativa	Islamabad	Seeds	Aqueous methanol	DPPH	Ali *et al.* (2020)
C. sativa	Gilgit-Baltistan	Whole plant	Ethyl acetate	ND	Rida *et al.* (2020)
C. sativa	Gilgit-Baltistan	Leaves	Aqueous	ABTS, DPPH, lipid peroxidation assay and TBARS	Hussain *et al.* (2021)
C. sativa	Gujranwala and Narowal	Seed	Hexane	DPPH	Nawaz *et al.* (2021)

ABTS; Azinobis-3-ethylbenzothiazoline-6-sulfonate, DPPH; 2,2-Diphenyl, 1-picrylhydrazyl hydrate, ND; Not defined, TAEC; Trolox equivalent antioxidant capacity

Table 5. Anticancer activities of Cannabinoids from *Cannabis* against different tumor types reported worldwide

Cancer type	Study model	Cell lines	Results	Reference
Breast cancer	*In vitro*	MCF-7 and EFM-19	Cell cycle arrest	De Petrocellis *et al.* (1998)
		MCF-7 and T-47D		Melck *et al.* (2000a, b)
		MDA-MB231 and MDA-MB436	Decreased Id-1 expression, reduced tumor	McAllister *et al.* (2007)
		EVSA-T, MDA-MB-231, MDA-MB-468, SKBr3, MCF-7 and T-47D	Blocked cell cycle progression and induced apoptosis,	Caffarel *et al.* (2006)
	In vivo (mouse)	BT474, MDA-MB-231, MCF-7 and SkBr3	Reduced tumor progression	Caffarel *et al.* (2010)
		MDA-MB-231	Reduced cell viability. Induced apoptosis and autophagy	Shrivastava *et al.* (2011)
	In vivo	MDA-MB231-luc-D3H2LN	Inhibits cell proliferation and invasion in culture and metastasis	Murase *et al.* (2014)
Colorectal cancer	*In vivo* (mouse); *in vitro*	DLD-1 and HT29	Apoptosis; reduced cell proliferation	Cianchi *et al.* (2008)
		ND		Aviello *et al.* (2012)
		Caco-2, HCT 116, HCEC and HEK-293		Borrelli *et al.* (2014)
		DLD-1 and HCT116		Romano *et al.* (2014)
Glioma	*in vivo* (mouse, rat); *in vitro*	C6	Decreased tumor size; apoptosis	Galve-Roperh *et al.* (2000)
		C6		Jacobsson *et al.* (2000)
		U87 and U373		Massi *et al.* (2004)
		C6 and C6.9		Sánchez *et* al. (1998, 2001)
		U87		Torres *et al.* (2011)
		SF-126 and U251	Synergic inhibition of cell proliferation	Marcu *et al.* (2010)
		U87-MG and T98G	Inhibition of proliferation and invasion	Solinas *et al.* (2013)
Liver cancer	*In vivo* (mouse); *in vitro*	HCC HepG2	Human HCC death via induction of autophagy	Vara *et al.* (2011)
Lung carcinoma	*In vivo* (mouse); *in vitro*	Lewis lung cells	Decreased tumor size; inhibition of cell growth	Munson *et al.* (1975)
	In vivo (mouse)	A549	Suppression of subcutaneous tumor growth and metastasis	Preet *et al.* (2008)
	In vitro	A549, H460 and H1792	Reduced migration of cells	Milian *et al.* (2020)

Lymphoma	*In vivo* (mouse); *in vitro*	Jurkat, Molt-4 and Sup-T1	Decreased tumor size; apoptosis	McKallip *et al.* (2002)
		EL-4, LSA and P815	Increased apoptosis and reduced cell viability	Sarfaraz *et al.* (2008)
Neuroblastoma	*In vitro*	CHP100 and U937 cells	Apoptosis	Maccarrone *et al.* (2000)
Ovarian cancer	*In vitro*	OVCAR3	Regulates cell proliferation	Piñeiro *et al.* (2011)
	In ovo	ND	Tumor growth inhibition	Fraguas-Sanchez *et al.* (2020)
Skin carcinoma	*In vivo* (mouse); *in vitro*	DV.C57, HaCa4, MCA3D and HaCat	Decreased tumor size; apoptosis	Casanova *et al.* (2003)
Pancreatic cancer		Capan-2, PANC-1, MIA PaCa-2 and BxPC-3	Decreased cell viability	Carracedo *et al.* (2006)
Prostate carcinoma	*In vitro*	PC-3	Apoptosis	Ruiz *et al.* (1999)
		LNCaP, DU145, and PC-3		Mimeault *et al.* (2003)
		DU-145		Melk *et al.* (2000b)
		LNCaP	Inhibition of cell growth	Sarfaraz *et al.* (2005)
		PC-3, DU-145 and LNCaP	Decreased cell viability	De Petrocellis *et al.* (2013)
		PC-3 and DU-145	Regulates cell proliferation	Piñeiro *et al.* (2011)
Uterus carcinoma		HeLa S_3	Inhibition of cell growth	Mon *et al.* (1978)
		HeLa		Blevins & Smith (1980)

ND = Not defined

Even though after obtaining a permit from federal government or provincial government, the cultivation of hemp will be allowed for scientific research, medical or industrial practices. If found defilement, it is illegal and a serious crime with detention with fine. This is predominantly factual in numerous tribal areas of Pakistan, where *Cannabis* occasionally is distributed in public markets leading to drug abuse. Beforehand the medical or industrial community can progress their research on *Cannabis,* its uses in treatment or by products manufacturing in industry, it has to evaluate and disseminate the knowledge, practices and attitudes of the community.

Table 6. Global production of hemp in 2018; adapted from Zhao et al. (2021)

Hemp producing country	Source of data	Area used (ha)
USA	USDA	36,422
Canada	Health Canada	31,537
North Korea	FAOSTAT	21,457
France		17,284
China		8791
Russia		7953
Chile		7046
Romania		2795
Ukraine		2613
Hungary		1860

Conclusion

There is a history of centuries that *Cannabis* has been used medicinally and *Cannabis* species are unquestionably among the primitive plant-based remedies known to humans. *C. sativa* especially is the most studied plant species because of significant therapeutic associations in its extracts as it contains pharmacologically and chemically diverse cannabinoids (CBDs). *Cannabis* species have long been used as folk traditional medicine in different regions of Pakistan and the compounds from *Cannabis* are used against, reactive oxygen species (ROS), cancer and microbial infections including both bacterial and fungal strains. Consequently, different phytochemical compounds isolated from *Cannabis* may have different pharmacological actions and therefore support the utilization of species infusions and/or decoctions as folk traditional medicine in Pakistan. It is believed that *Cannabis* (hemp) legalization in Pakistan could upsurge the National revenue by exporting the raw material or *Cannabis* based by-products. It is recommended that the legalization of *Cannabis* (hemp) in Pakistan should be complemented with wide-ranging approaches to disseminate medicinal importance and keep the hemp containing psycho-active compounds (THC) out of the minor's hands and provide knowledge and awareness on both of its medicinal and harmful effects.

Declarations

List of abbreviations: ABTS = Azinobis-3-ethylbenzothiazoline-6-sulfonate; AJK = Azad Jammu and Kashmir; ANF = Anti-narcotics force; CBDs = Cannabinoids; CBL = Cannabicyclol; *C. sativa = Cannabis sativa*; DPPH = 2,2-Diphenyl, 1-picrylhydrazyl hydrate; D9-THC = Trans-Δ-9-tetrahydrocannabinol; GB = Gilgit-Baltistan, GC-MS = Gas chromatography-Mass spectroscopy; HPLC = High performance liquid chromatography; KPK = Khyber Pakhtunkhwa; ND = Not defined; PNCB = Pakistan Narcotics Control Board; ROS = Reactive oxygen species; TFC = Total flavonoid contents; THC = Tetrahydrocannabinol; TPC = Total phenolic contents, TAEC = Trolox equivalent antioxidant capacity; UNODC = United Nations Office on Drugs and Crime; UV-Spectro = Ultraviolet visible spectrophotometer

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