Chapter 2
Hemp Varieties: Genetic and Chemical Diversity

Varsha Mishra, Khashti Dasila, Mithilesh Singh, and Deepika Tripathi

Abstract *Cannabis sativa* (hemp) as multifunctional crop have traditional application as fiber, food, paper, textile and pharmaceutical potential as inflorescences and seed as sources of exciting bioactive secondary metabolites. The Genus *Cannabis* is the only producer of phytocannabinoids. Extensive studied have been made to describe the origin history, geographical ranges and genetic identity of the *Cannabis* species but it remains obscured to date. Various high through put genetic marker have been studied to assess the genetic diversity in hemp varieties. Studies also indicated that domestication origin affects the genetic groups of hemp which further consequences on the chemical diversity of the cannabis. Chemotaxonomy using chemical markers also played a crucial role in differencing and allocating the *Cannabis* taxa. Cannabinoids ratio and terpene composition are the major marker to play an important role in studying chemical diversity of *Cannabis* sp. *Cannabis* genus is the only source of phytocannabinoids the dominant chemical class. Other than cannabinoids terpene and non-cannabinoid phenolic compounds also contribute in the chemical diversity of the species. The vast array of phytochemicals presents in the genus have potential application in pharmaceutical industries. However, due to its legalization status very limited study on its chemical and genetic diversity have been done. Therefore, the species needs attention to explore its commercial value.

Keywords *Cannabis sativa* · Hemp · Phytocannabinoids · Genetic diversity · Chemotaxonomy

2.1 Introduction

Cannabis is an erect, annual, dioecious and economically important aromatic medicinal herb belonging to the cannabaceae family (Pellati et al. 2018). The origination of *cannabis* was believed to be from Central Asia about ~500 BC (Farag and Kayser

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2017). Plants belongs to this genus are well grown in wet land sites and near water bodies, where the concentration of nitrogen are found high (Small 2015). *Cannabis* is highly used and described genus in Ayurveda to provide various pharmacological bioactive compounds and benefits. Now a days, people take interest in this multipurpose plant due to the presence of high content of various nutrients along with bioactive therapeutic compounds having analgesic, anti-spasmodic, anti-tumour, anti-inflammatory, anti-oxidant, antineoplastic**,** neuro-protective, immunosuppressive, anti-nociceptive, antiepileptic, and anti-depressant properties (Carchman et al. 1976; Ameri et al. 1999; Callaway 2004; Gomes et al. 2008; Appendino et al. 2011) Cannabinoids, the major fundamental phytoconstituent of this genus which are chemically a unique class of terpenophenolic compounds having pharmaceutical potential such as anti0inflammatory, anti-cancer, antimicrobial, anti-arthritic, neuro antioxidative, etc. (De Petrocellis et al., 2011). Apart from this, hemp is also used by the mankind as natural fibres in the textile industry and as seed oil in the cosmetic production (Gautam et al. 2013; Clarke and Merlin 2013; Russo et al. 2008; Farag and Kayser 2017). Hemp use as a suitable eco-friendly option for phyto-remediation and bio fuel production has also been reported (Kumar et al. 2017).

Hemp is a highly variable species in plant system and it has been a matter of debate that whether the genus *Cannabis* having one species or more than one species (Chandra et al. 2017). According to Hazekamp and Fischedick (2012), *Cannabis* is a monotypic genus and consists a single species namely *Cannabis sativa* (described by Leonard Fuchs in the sixteenth century). Approximately 700 different varieties/cultivars of *Cannabis* have been identified and distinguished by the plant breeders and recreational users due to the results of centuries of breeding and selection. However, it is unclear whether or not these cultivars reflect any relevant differences in chemical composition.

2.2 Varieties of Hemp

With the course of time, different varieties of hemp have been evolved as the result of plant breeding and selection programme. Till so far, no in-dept study on the monospecific or/and polyspecific character of genus *Cannabis* has been made. According to the (United Nations Office on Drugs and Crime (UNODC) hemp is divided into three different categories like (a) fiber hemp (b) oil seed hemp and (c) drug hemp (Farag and Kayser 2017). Similarly, Schultes et al. (1974) also differentiated this genus into three species such as *C. sativa* L.*,* *C. indica* L.*,* and *C. ruderalis.* However, many reports are available on *Cannabis* is monotypic genus that consist only a single species *C. sativa* (Beutler and Dermarderosian 1978; Hoffmann 1961). Small and Cronquist (1976) divided the monotypic species *C. sativa* into the subspecies 'sativa' and 'indica' each with two different variants i.e., domesticated (*C. sativa* subsp. *sativa* var. *sativa* and *C. sativa* subsp. *indica* var. *indica*) and wild varieties (*C. sativa* subsp. *sativa* var. *spontama* and *C. sativa* subsp. *indica* var. *kafiristanica*). In last

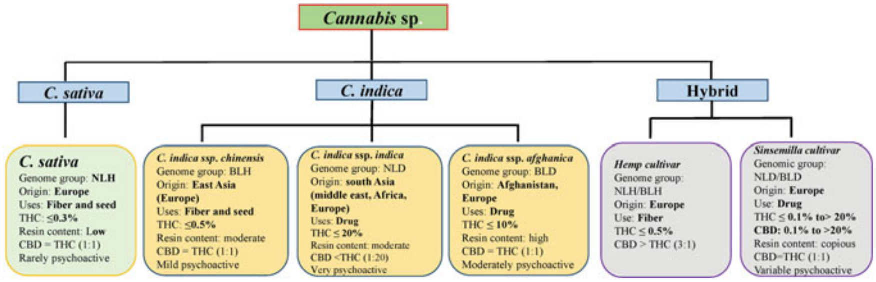

Fig. 2.1 Modern *Cannabis* taxonomy given by Clarke and Merlin (2016). Abbreviation: NLH = narrow leaflet hemp; BLH = broad leaflet hemp; NLD = narrow leaflet drug; BLD = broad leaflet drug; CBD = cannabidiol, and THC = delta-9-tetrahydrocannabinol

two decades, various new hybrid varieties have been also developed like 'Super-sativa', 'Bedrocan', 'Bedrobinol', and 'Bediol' etc. (Clarke and Watson 2002; de Meijer 2004; Flemming et al. 2007). Many *Cannabis* hybrid varieties and some pure strains have been now commercialized by many private farms and ~20 strains are well defined for the cultivation of Hemp. A large number of plant breeders cultivate fiber hemp variety with the target to reduce THC concentration (de Meijer 1995). During the origination process of plant, particularly Hemp opened the path to hybridization and leads the development of thousands of cultivars. Small (2015) stated that there is a serious taxonomic issue to classify the different strains of *Cannabis* and divide the *C. sativa* L. species into 3 subspecies or variants such as '*sativa*' (industrial cannabis/hemp having a limited amount of tetrahydrocannabinol or THC), '*indica*' (medicinal cannabis/marijuana producing principally THC), and '*ruderalis*' (known for wild hemp strains). Clarke and Merlin (2016) referred *C. ruderalis* as ancestor of two modern *Cannabis* sp. (*C. sativa* and *C. indica*), originated from central Asia. The recent taxonomy of *Cannabis* was given by Clarke and Merlin (2016) as presented in Fig. 2.1.

2.3 Geographical Distribution

Cannabis has usually a wide range of geographical and ecological distribution. It is grown worldwide except Antarctica, in a broad range of environment from sub-arctic to temperate and tropical from sea level to over 3000 m elevation (Clarke and Merlin 2013; Glanzman 2015). This genus is believed to have originated in the Northwest Himalayas and widely distributed in the range of Africa. Small and Cronquist (1976) reported that genus *Cannabis*, geographically distributed towards latitude 30°N (North) and 60°N (South) (Hillig 2005).

Clarke and Merlin (2013), reported that *Cannabis* is distributed worldwide by humans for multiple purposes. According to the authors, the putative ancestor of *Cannabis* is originated in Central Asia. It is hypothesized that *Cannabis* distributed

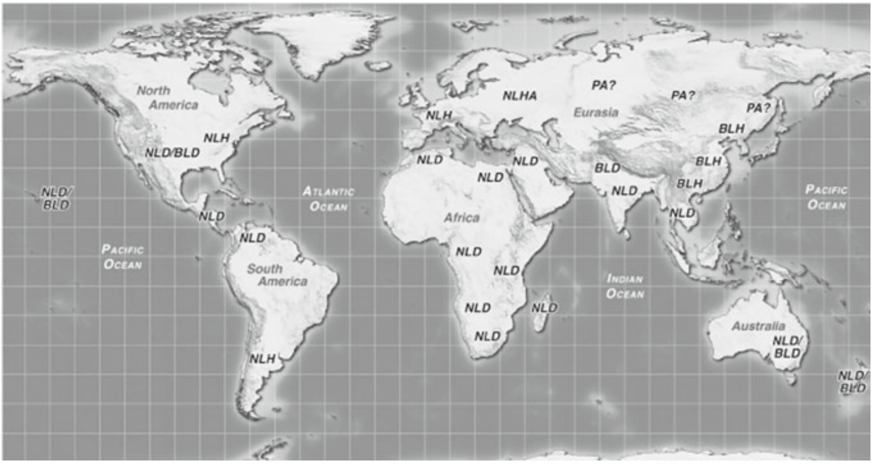

Fig. 2.2 Graphical distribution of *Cannabis* subspecies (Clarke and Merlin 2016)

into new geographical areas and evolved into 4 taxonomic groups along with gene pools as *Cannabis sativa* narrow leaflet hemp (NLH), *C. indica* broad leaflet hemp (BLH), *C. indica* narrow leaflet drug (NLD), *Cannabis indica* spp. *afghanica* broad leaflet drug (BLD). Based on the broad taxonomic groups the worldwide distribution of *Cannabis* is given in Fig. 2.2.

2.4 Genetic Diversity

Identification of functional gene variation and trait mapping is important step for understanding toward the evolutionary and functional aspects of *Cannabis.* Hemp possesses diploid genome ($2n = 20$) with difference in sizes as 818 Mb for female and 843 Mb for males (Sakamoto et al. 1998). Inspite of being restricted due to legalization stautus various authors attempted to study the hemp genetic for various traits like fiber quality, sex determination, sex expression, assessment of population diversity, and genetic relatedness between strains using various genetic tools etc. The genomic markers used for various trait mapping in hemp species are described in Table 2.1.

Various issues regarding naming, breeding and quality control arises in hemp varieties cultivation. According to Lynch et al. (2016) and Schwabe et al. (2019) traditional classification did not determine the genetic relationship in drug-type and fibre-type *Cannabis* species i.e., '*indica*' and '*sativa*'. Both drug-type and fiber-type *C. sativa* plants are genetically different and have been used for breeding practices for various purposes (van Bakel et al. 2011; Sawler et al. 2015; Lynch et al. 2016; Vergara et al. 2021). Previous studies usually focused on *Cannabis* species having high CBD/low THC but sometime these are closely related to marijuana

Table 2.1 Genetic markers/tools used for the study genetic diversity studies in *Cannabis sativa* (Hamp)

S. no	*Cannabis* sp.	Genetic markers/tool used	Application	Reference
1	*Cannabis sativa*	Random amplified polymorphic DNA (RAPD)	Genetic variation between cultivars/accession	Faeti et al. 1996
2	*Cannabis sativa*	Amplified fragment length polymorphism (AFLP)	Genetic variation hemp and marijuana	Datwyler and Weiblen 2006
3	*Cannabis sativa*	Inter simple sequence repeat (ISSR)	genetic relatedness in drug- type and hemp- type	Hakki et al. 2007
4	*Cannabis sativa*	Organelle DNA (chloroplast and mitochondria)	Differentiation between hemp and marijuana population	Gilmore et al. 2007
5	*Cannabis sativa*	Genomic libraries	Fiber quality	Van den Broeck et al. 2008
6	*Cannabis sativa*	Genomic libraries	Transcriptomes analyse	van Bakel et al. 2011; Laverty et al. 2019
7	*Cannabis sativa*	Amplified fragment length polymorphism (AFLP)	Population genetic diversity	Hu et al. 2012
8	*Cannabis sativa*	Single-nucleotide polymorphisms (SNPs)	Genetic diversity and population structure among hemp and marijuana	Sawler et al. 2015
9	*Cannabis sativa*	amplified fragment length polymorphism (AFLP)	Effect of Gene duplication and divergence on cannabinoid production	Weiblen et al. 2015
10	*Cannabis sativa*	amplified fragment length polymorphism (AFLP)	sex expression in monoecious and dioecious hemp	Faux et al. 2016
11	*Cannabis sativa*	Inter simple sequence repeat (ISSR)	Genetic relationship between strains	Punja et al. 2017
12	*Cannabis* sp. (Hemp and marijuana)	Short tandem repeats (STR)	Characterization of drug vs. non-drug *Cannabis*	Dufresnes et al. 2017

(continued)

Table 2.1 (continued)

S. no	*Cannabis* sp.	Genetic markers/tool used	Application	Reference
13	*Cannabis sativa*	Genotyping-By-Sequencing (GBS)	Genetic diversity and population structure in Iranian cannabis germplasm	Soorni et al. 2017
14	*Cannabis sativa*	Inter simple sequence repeat (ISSR)	Genetic relationship among strains	Punja et al. 2017
15	*Cannabis* sp.	Simple sequence repeat (SSR)	Genetic and population structure	Zhang et al. 2020
16	*Cannabis* sp.	Single-nucleotide polymorphisms (SNP)	Population structure, phylogenetic relationship, population genetics	Henry et al. 2020
17	*Cannabis sativa*	next generation sequencing and nanoHPLC mass spectrometry	Proteomic and metabolomic analysis	Jenkins and Orsburn 2020; Conneely et al. 2021

species (Grassa et al. 2021). However, both types have been practicing to bred for specific compound production like cannabinoids and terpenoids. *Cannabis* genetics evolving with focus on *C. sativa* marijuana-type as compared to CBD-type hemp (Lynch et al. 2016; Vergara et al. 2021, Johnson and Wallace 2021).

2.5 Chemical Diversity in *Cannabis Sativa*

Apart from being controversial crop for various issues related to taxonomic status, origin, morphological and ecological diversity, *Cannabis* exhibited extensive phytochemical diversity in particular reference to cannabinoid and terpenoid (Hillig and Mahlberg 2004). Phytocannabinoids are the dominant chemical class of genus *Cannabis*. Cannabinoids are terpenophenolic compounds which are chemically associated terpenes with its ring structure derived from C10 monoterpene subunit i.e., geranyl pyrophosphate. Geranyl pyrophosphate are the biogenetic origin of cannabinoids (Hanus et al. 2016). Two independent pathways namely cytosolic mevalonate and plastidial methylerythritol phosphate (MEP) are responsible for phytoterpene biosynthesis. MEP pathway is reported for the biosynthesis of the cannabinoid terpenoid moiety biosynthesis (Sirikantaramas et al. 2007; ElSohly et al. 2017). The cannabinoids accumulated in cannabis plant as cannabinoid acids and non-enzymatically decarboxylated into their neutral forms during storage (Small 2015).

Radwan et al. (2021) reviewed the phytochemistry, isolation, identification and structural elucidation of more than 500 constituents including cannabinoids and non-cannabinoids class of C. *sativa*. To date different secondary metabolites class of *C. sativa* were presented in Table 2.2. The chemical structures of cannabinoids and terpenoids compounds are presented in Figs. 2.3 and 2.4.

Table 2.2 Chemical diversity of *Cannabis sativa* (Radwan et al. 2021)

Chemical class	No. of compound reported	Analytical techniques used for identification
Total cannabinoids	*125*	Column chromatography, Thin Layer Chromatography (TLC), Vaccum Liquid Chromatography (VLC), Solid Phase extraction (SPE), Ultraviolet–visible infrared spectroscopy (UV IR), Nuclear Magnetic Resonance (NMR) spectroscopy (1D&2D), UV spectroscopy, Mass spectrometry (MS), semi preparative (Riverse phase- High performance Liquid Chromatography (RP-HPLC), Gas Chromatography Mass spectrometry (GCMS), X-ray analysis and High Resolution mass spectrometry (HRMS)
Δ9-THC type	25	
Δ8-THC type	5	
CBG type	16	
CBC type	9	
CBD type	10	
CBND type	2	
CBE type	5	
CBL type	3	
CBN type	11	
CBT type	9	
Miscellaneous types	30	
Total non-cannabinoids	*400*	
Non cannabinoid phenol	*76*	Silica gel column chromatography, TLC, VLC, 1H and 13C NMR and MS, X-ray crystallography, EIMS, flash chromatograph, HPLC, High resolution electrospray ionisation mass spectrometry (HR-ESIMS), and ESI–MS spectroscopy, GC–MS and RP-HPLC, HPLC–DAD-MS
Spiro-Indans	16	
Dihydrostilbenes	12	
Dihydrophenanthrenes	7	
Simple phenol	*7*	
Flavonoids	34	
Total Terpenes	*120*	Capillary gas chromatography, GC–MS analysis, GC-FID, Spectral data comparison
Monoterpenes	61	
Sesquiterpenes	51	
Diterpenes	2	
Triterpenes	2	
Miscellaneous terpenes	4	
Alkaloids	*2*	Silica gel chromatography, TLC and X-ray crystallography,

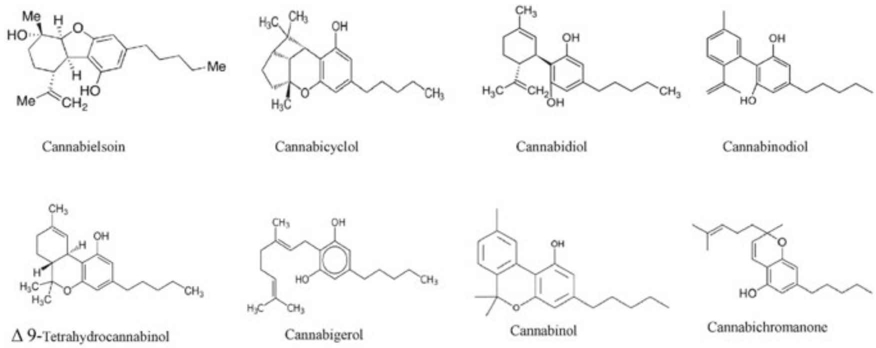

Fig. 2.3 Cannabinoids compounds in hemp

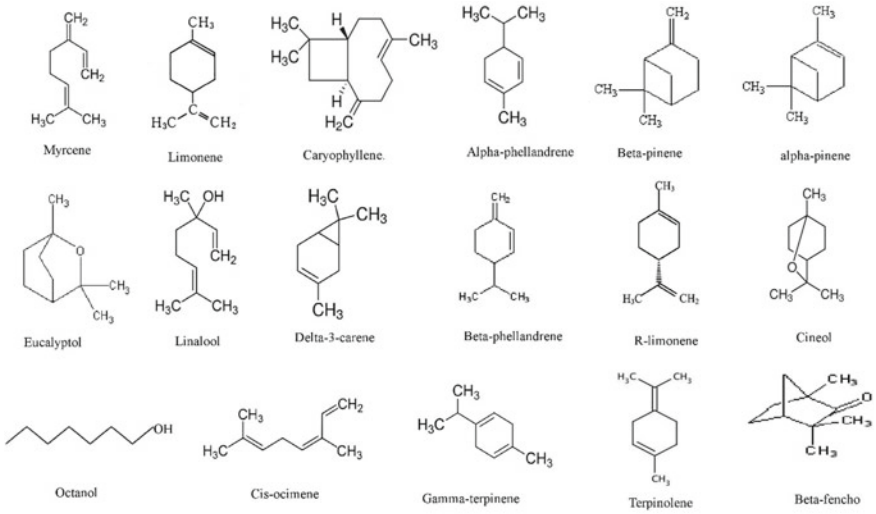

Fig. 2.4 Terpenoids compounds of hemp

2.6 Chemotaxonomic Classification of Hemp

Chemotaxonomy/chemosystematics is used to classify according to confirmable differences and similarities in their biochemical compositions. According to Small (1979a) amount of THC in *Cannabis* is essential for taxonomic characterization. Gas chromatography-flame ionization detection (GC-FID) is commonly used techniques to differentiate *indica* strains from *sativa* strains on the basis of THC content (Small and Beckstead 1973a, Small et al. 1975; Small and Cronquist 1976). Numerous phytochemical markers used for chemotaxonomic classification of *Cannabis* species/varieties are presented in Table 2.3. Based on the quantitative difference in the cannabinoids ratio of tetrahydrocannabinol acid (THC), cannabinol

Table 2.3 Analytical methods used for chemotaxonomic classification and phytochemical quantification of *Cannabis* sp.

S. no	*Cannabis* sp.	Chemotaxonomic markers	Analytical methods applied	Inferences	Reference
1	*C. sativa and C. ruderalis*	Cannabidiol (CBD) and Cannabichromene (CBC)	Gas chromatograph with flame ionization detection (GC/FID)	Cannabinoid content in *Cannabis* species is genetically control and monotypic evidences of *Cannabis* genus	Beutler and Marderosian 1978
2	157 *Cannabis* germplasm	Cannabinoids ratio	GC/FID	Based on THC/CBD ratio authors supported two-species concept of *Cannabis* as if < 25% (*C. sativa*) and > 25% (*C. indica*)	Hillig and Mahlberg 2004
3	*C. sativa* (11 varieties)	Monoterpenoids, sesquiterpenoids	GC/FID, Gas Chromatograph –Mass Spectroscopy (GC–MS)	This methodology is useful for both chemotaxonomic discrimination of *Cannabis* varieties and quality control of plant material	Fischedick et al. 2010
4	*Cannabis* sp. (40 samples)	Gamma terpinene (terpenoids) THC (cannabinoids)	GC, GC–MS	The cannabinoids and terpenoids, present in high concentrations in Cannabis flowers, are the main candidates	Hazekampa and Fischedick 2012
5	*C. indica* and *C. sativa*	Cannabinoids and terpenoids	UFLC (Cannabinoid), GC-FID (Terpenoids)	Studied *Cannabis* cultivars are not distinctly different chemotypes	Elzinga et al. 2015

(continued)

Table 2.3 (continued)

S. no	*Cannabis* sp.	Chemotaxonomic markers	Analytical methods applied	Inferences	Reference
6	*Cannabis* OG strain and Kush strain	Cannabinoids and terpenoids	UFLC (Cannabinoid), GC-FID (Terpenoids	OG strain have relatively high levels of terpenoids than kush strain	Elzinga et al. 2015
7	*Cannabis* (460 accessions)	beta-pinene (Monoterpenes) alpha-humulene (sesquiterpene) CBD (cannabinoids)	GC	Chemical constituents of *Cannabis* may act as markers for differentiation between '*indica*' and '*sativa*'	Hazekamp et al. 2016
8	*C. sativa* (2 varieties)	Cannabinoids profile, terpene composition, polyunsaturated fatty acids content, and favourable protein profile	HPLC–MS, SDS-PAGE LC–MS/MS, HS-SPME GC–MS, and GC-FID	Phytochemical and Ecological Analysis	Pavlovic et al. 2019
9	*Cannabis* (21 varieties) Leaf, inflorescence, stem, bark, and roots	Cannabinoid ratio, terpenoid, flavonoids, sterols, and triterpenoids	GC–MS (mono and sesquiterpenoids, flavonoid) HPLC	Predominant number of identified chemotype markers (with or without THC and CBD) can use in chemical fingerprints for quality standardization or strain identification	Jin et al. 2021

(CBN) and cannabidiol (CBD), in the ratio of (THC) + (CBD)/(CBN) *C. sativa* classified in three different chemical phenotypes. If THC/CBD > 1 phenotype I (drug-type), THC/CBD = 1 phenotype II (intermediate type) THC/CBD < 1 phenotype III (fibre- type or hemp) (de Meijer et al. 2003; De Backer et al. 2009; Galal et al. 2009).

Health benefits associated to cannabinoids and non-cannabinoids have been presented in Table 2.4. The biological properties related to cannabinoids mainly associated with human endocannabinoid system. The system consists of two G-protein coupled receptors i.e., CB1 and CB2 along with two ligands. The system

Table 2.4 Pharmaceutical potential of cannabinoids and non-cannabinoids compounds of *Cannabis sativa*

Secondary metabolite class	Pharmaceutical application	References
Cannabinoids type		
Δ-9 tetrahydrocannabinol	Anti-inflammatory, anti-cancer, analgesic, muscle relaxant, neuro-antioxidative and anti-spasmodic activities	De Petrocellis et al. 2011
Cannabidiol (CBD)	anti-inflammatory, antischizophrenic, antiepileptic, anti-bacterial, anti-anxiety, anti-nausea, anti-arthritic, anti-psychotic, and immunomodulatory properties	Mechoulam 2005, ElSohly and Slade 2005, Burstein 2015, Appendino et al. 2008
Cannabigerol (CBG)	Antimicrobial, analgesic, antileishmanial, and inflammatory bowel disease	Mechoulam and Gaoni 1965; Radwan et al. 2008; Borrelli et al. 2013
Cannabichromene (CBC)	Anti-inflammatory, sedative, analgesic, anti-bacterial and antifungal properties	Shoyama et al. 1972, Delong et al. 2010; Davis and Hatoum 1983, Eisohly et al. 1982
Terpenes		
Monoterpenes	Anti-cancer, anxiolytic, immunostimulant, anti-inflammatory, analgesic and anticonvulsant	Komori et al. 1995; Cleemput et al. 2009; Russo 2011
Sesquiterpenes	Anti-inflammatory and gastric cytoprotector activities	Singh and Sharma 2015
Triterpenes	Anti-bacterial, anti-fungal, anti-inflammatory and anti-cancer properties	Vázquez et al. 2012; Moses et al. 2013
Phenolic Compounds	Antioxidants, antidiabetic, antitumorigenic and anti-obesity activities, anti-inflammatory, anti-cancer and neuro-protective properties, oestrogenic properties, cytotoxic activities	Andre et al. 2010; Wang and Kurzer 1998; Werz et al. 2014; Sun et al. 2014; Cui-Ying et al. 2002

mainly thought to regulatory role in various physiological processes including pain-sensation, mood, apatite, memory, inflammation and metabolic pathways. CB1 receptors also present on cells of gastrointestinal, adrenal, lung, heart and immune systems however, CB2 receptors exerted immunomodulary effect (Andre et al. 2016). Similarly, terpenes and phenolic compounds linked to *Cannabis* sp. possesses various biological activities (Table 2.4).

2.7 Conclusion and Future Prospects

Despite of being controversial status of genus *Cannabis,* it is cultivated worldwide for its high pharmaceutical and industrial potential. Although, chemotaxonomy plays an important role to differentiate various varieties of hemp, but, the origination history and genetic diversity of *Cannabis* species still remain largely unexplored. As identification of elite chemotype is essential for production of cannabinoid for industrial use, it is paramount to first study genetic and chemical diversity of the plant in details. Considering high medicinal value of Indian varieties, it is essential for the scientific community to start working on this important medicinal agriculture crop. This will not only facilitate in drug discovery programme but can be proved as a boon for the marginal communities of the Himalayan region where this plant is growing as a weed.

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