# Polypharmacological Properties and Therapeutic Potential of β-Caryophyllene: A Dietary Phytocannabinoid of Pharmaceutical Promise

Charu Sharma<sup>a</sup>, Juma M. Al Kaabi<sup>a</sup>, Syed M. Nurulain<sup>b</sup>, Sameer N. Goyal<sup>c</sup>, Mohammad Amjad Kamal<sup>d,e</sup> and Shreesh Ojha<sup>f\*</sup>

<sup>a</sup>Department of Internal Medicine, College of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, UAE; <sup>b</sup>Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan; <sup>c</sup>Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule, MS, India; <sup>d</sup>King Fahd Medical Research Center, King Abdulaziz University, P.O. Box 80216, Jeddah, Kingdom of Saudi Arabia & <sup>e</sup>Enzymoics, 7 Peterlee Place, Hebersham, NSW 2770, Australia; <sup>f</sup>Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, UAE



Abstract: Background: β-Caryophyllene (BCP) is a natural bicyclic sesquiterpene abundantly found in essential oils from various spices, fruits and medicinal as well as ornamental plants. It is approved by United States Food and Drug Administration and European agencies as food additive, taste enhancer and flavoring agent and termed as

a phytocannabinoid. *Methods:* All the available literature on BCP and its synonyms were collected through different literature databases. *Results:* BCP was found to elicit a full agonist action on cannabinoid type 2 (CB2) receptors, a G-protein coupled receptor representing important therapeutic target in several diseases. Activation of CB2 receptors notably appeared devoid of psychotropic adverse effect of cannabinoids contrary to the CB1 receptors. In addition, it activates peroxisome proliferated activator receptors (PPARs) isoforms; PPAR- $\alpha$  &- $\gamma$  and inhibits pathways triggered by the activation of toll like receptor complex; CD14/TLR4/MD2, reduce immune-inflammatory processes and exhibit synergy with  $\mu$ -opioid receptor dependent pathways. Additionally, it found as potent antagonist of homomeric nicotinic acetylcholine receptors ( $\alpha$ 7-nAChRs) and devoid of effects mediated by serotonergic and GABAergic receptors. It also modulates numerous molecular targets by altering their gene expression, signaling pathways or through direct interaction. Various pharmacological activities such as cardioprotective, hepatoprotective, gastroprotective, neuroprotective, nephroprotective, antioxidant, anti-inflammatory, antimicrobial and immune-modulator have been reported in experimental studies. It has shown potent therapeutic promise in neuropathic pain, neurodegenerative and metabolic diseases. *Conclusion:* The present review provides a comprehensive insight of pharmacological and therapeutic potential of BCP, its molecular mechanism and signaling pathways in different pathological conditions. The review also examines the possibility of its further development as a novel candidate for various pathologies considering the polypharmacological and multifaceted therapeutic properties potential along with favorable oral bioavailability, lipophilicity and physicochemical properties.

**Keywords**: β-Caryophyllene, CB2 receptor agonist, AM630, dietary phytocannabinoids, trans-Caryophyllene, phytochemicals, PPAR isoforms, CB2 receptors.

# **1. INTRODUCTION**

Beta-caryophyllene (BCP) is a volatile bicyclic sesquiterpene lactone compound abundantly found in the essential oils of many dietary and edible plants including spices such as cloves (Syzygium aromaticum), oregano (Origanum vulgare), cinnamon (Cinnamomum spp.), hemp (Cannabis sativa), rosemary (Rosmarinus officinalis), hops (Humulus lupulus) and black pepper (Piper nigrum), germander (Teucrium spp.) and Copaiba oil (Copaiba spp.) [1-5]. It is one of the important components, which contributes to the piquancy taste to the spice, pepper. BCP shows resemblance with structure and properties of cannabinoid related molecule therefore termed as 'phytocannabinoid' [6]. Due to extensive occurrence across the dietary plants, it's a common component to human dietary regimes and consumed either as flavor or food preservative or additive, thus often quoted as a 'dietary cannabinoid' [6]. It is one of the common ingredients of candies, confectionaries, chewing gum, toothpastes, beverages, pharmaceuticals and cosmetic products such as perfumes, shampoos and soaps for its aroma and in pesticides [7,8]. BCP is designated as "generally recognized as safe" by several other regulatory agencies [6,9,10]. Recent approval of BCP as food additives and flavoring agent for commercial use in foodstuffs and cosmetics by the USFDA and European Food Safety Authority (EFSA) generated noteworthy interest among scientific community to explore it for its additional possible therapeutic benefits [6,11]. It exhibited significant therapeutic potential in numerous diseases due to multiple pharmacological properties to name a few like antioxidant, anti-inflammatory [6], antimicrobial [12], anticancer and chemopreventive [13].

The present review presents a comprehensive insight of the polypharmacological properties and therapeutic potential of BCP, its molecular mechanism and signaling pathways in different pathological conditions. Along with polypharmacological properties and multifaceted therapeutic potential, the review also illustrates further advancements reviewing its physicochemical properties. The natural availability, favorable bioavailability and lipophilicity make BCP a novel candidate for pharmaceutical development and clinical applications.

# 2. SOURCES AND MEDICINAL CHEMISTRY OF $\beta$ -CARYOPHYLLENE

BCP is mainly present in the essential oils obtained from different parts or whole plant mainly aromatic plants. It has been reported to exist in more than one thousand plants that provide extensive natural availability and accessibility. It is also one of the significant constituents (about 35%) in the essential oil of marijuana (*Cannabis sativa L.*), which is considered to be a major traditional source of plant derived cannabinoids or phytocannabinoids [6,14-16]. Till date, about 1500 papers are available through different literature

<sup>\*</sup>Address correspondence to this author at the Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, UAE; E-mail: shreeshojha@uaeu.ac.ae

No.	Plant name	Parts	% BCP*	References
1	Acanthospermum hispidum	Oil	35.2	[17]
2	Callicarpa integerrima	Leaves	33.7	[18]
3	Canarium parvum Leen	Stem	30.4	[19]
4	Copaifera multijuga	Trunks	36.0	[20]
5	Copaifera multijuga Hayne	Trunks	57.5	[21]
6	Copaifera reticulata Ducke	Trunks	40.9	[21]
7	Croton heliotropiifolius	Oil	35.8	[22]
8	Euphorbia cotinifolia	Leaves	39.3	[23]
9	Glechon marifolia	Oil	32.2	[24]
10	Guarea humaitensis	Leaves	33.3	[25]
11	Hyptis pectinata	Oil	54.1	[26]
11	Lantana montevidensis	Leaves	31.5	[27]
12	Leucas indica	Aerial parts	51.1	[28]
13	Leucas aspera	Aerial parts	34.2	[29]
14	Melampodium divaricatum	Aerial parts	56.0	[30]
15	Mydocarpus fraxinifolius	Leaves	63.0	[31]
16	Murraya paniculata	Leaves	30.0	[32]
17	Myrica rubra	Leaves	89.9	[33]
18	Newbouldia laevis	Leaves	36.0	[34]
19	Peristrophe bicalyculata	Oil	33.9	[35]
20	Phoenix dactylifera	Buds	44.2	[36]
21	Pinus halepensis	Oils	30.0	[37]
22	Piper guineense, white	Fruits	51.7	[38]
23	Piper guineense, black	Fruits	57.6	[38]
24	Piper Nigrum	Fruits	70.4	[39]
25	Piper guineense, black	Oil	30.33	[40]
26	Piper duckei	Leaves	30.0	[41]
27	Plectranthus rugosus	Aerial parts	36.0	[42]
28	Plectranthus neochilus	Leaves	30.0	[43]
29	Stachys cretica	Aerial parts	51.0	[44]
30	Tabernaemontana catharinensis	Leaves	56.9	[45]
31	Teucrium chamaedrys	Aerial parts	47.6	[46]
32	Teucrium polium	Aerial parts	52.0	[47]
33	Teucrium flavum	Aerial parts	32.5	[47]
34	Teucrium divaricatum	Aerial parts	30.1	[48]
35	Valeriana alliariifolia	Aerial parts	38.9	[49]
36	Veronia albicans	Aerial parts	34.3	[50]

Table 1.	Percentage occurrence	of β-Caryophyllene	(BCP) in	different parts of	f the plants.
----------	-----------------------	--------------------	----------	--------------------	---------------

\*Plants containing  $\beta$ -Caryophyllene (BCP) equal to 30% or more.

databases including Scopus, Google Scholar, EMBASE and Pubmed/Medline. All these available literature either reporting the characterization of BCP as a constituent or evaluating the activity of BCP are included in this review. Those medicinal plants which were recognized to contain the amount of BCP equal to or than 30% of total phytoconstituents (about one third of the all phytoconstituents is BCP) are presented in Table 1.

Like several other phytoconstituents, the content of BCP varies from different geographical regions and biodiversity zones [51]. The plants containing BCP show noticeable variation in the amount of BCP produced as a secondary metabolite among different varieties grown in different habitats or different ecotypes within a particular species [52]. The plants adapted to diverse climates including altitudes, which exhibit distinct variability to the varying temperatures and numerous metabolic and physiological adaptations to the unfavorable environmental conditions. This is demonstrated by the appearance of huge variability in terpene profile of the Pinus heldreichii population from Galičica (Scardo-Pindic mountain system) was similar to those of the populations from Lovćen, Zeletin, Bjelasica, and Zlatibor-Pešter (belonging to the Dinaric Alps). But, the same species, Pinus heldreichii grown in Balkan-Rhodope Mountain lacks BCP [53]. Usually, in plants, BCP is mostly concentrated in the aerial parts, leaves, flowers and florescence and in traces in roots, rhizomes, stems and barks of different plants [54-56].

Chemically, BCP is known as (trans-(1R,9S)-8-Methylene-4,11,11-trimethylbicyclo (7.2.0) undecene and also recognized by several other synonyms such as caryophyllene, trans-caryophyllene, (-)-trans-caryophyllene,  $\beta$ -(E)-caryophyllene and L-caryophyllene (Fig. 1). Optically,  $\beta$ -Caryophyllene and iso-caryophyllene are trans- and cis- double isomers respectively, while  $\alpha$ -humulene is a ring-opened isomer. In plants, the BCP is generally found in their essential oils as a mixture with iso-caryophyllene or  $\alpha$ -humulene [57].

The physicochemical properties of BCP and its accession number with different regulatory and indexing authorities are presented in Table **2**. Naturally, BCP present in plants as a mixture of two pharmacologically-active isomers E-BCP and Z-BCP, together with substantially inactive sesquiterpenes such as alpha-humulene and derivatives such as BCP oxide. Though, natural sources include a greater proportion of E-BCP than Z-BCP [6].

BCP in different forms in foods and cosmetics is being used since 1930, however the first total synthesis was carried out by Corey and colleagues in 1964 [10]. The naturally occurring terpenes in plants produced as secondary metabolites were synthesized by the participation of several enzymes such as terpene synthases (TPSs) which accept the ubiquitous prenyl diphosphates geranyl diphosphate, farnesyl diphosphate and geranylgeranyl diphosphate as substrates for synthesis. These were further converted into the different varieties of mono-, sesqui- and diterpene skeletons, respectively [58]. The pathways for the synthesis of terpenes were studied extensively and manipulated in order to enhance the synthesis of desired compound for commercial production to meet the demand and supply. Likewise, the spatiotemporal regulation of biosynthesis of sesquiterpene especially BCP in Arabidopsis thaliana during flowering stage was showed by modulating expression of the sesquiterpene synthase gene TPS21 regulated by microRNA156 (miR156)-targeted squamosa promoter binding protein-like transcription factor. Manipulating the sesquiterpene synthase gene signifies the link between developmental timing and sesquiterpene production and demonstrates the potential strategies to engineer plants for accelerated growth with enhanced production of terpenoids [59].

In order to enhance the production of BCP and its derivatives, several genetic manipulations were shown promising in the medicinal plants containing BCP as one of the major components [60,61].

# Table 2. The physicochemical properties of β-Caryophyllene.

Chemical name	β-Caryophyllene
Synonyms	(E)-beta-caryophyllene,
	trans-Carvonhyllene
	isocaryophyllene,
	L-Caryophyllene
PubChem CID	5281515
InChI Key	NPNUFJAVOOONJE- FUGXAQUSA-N
CAS Number	87-44-5
Beilstein Number	2044564
MDL	MFCD00075925
ECHA EC Number	201-746-1
FDA UNII	BHW853AU9H
JECFA number	1324
CoE Number	2118
FEMA No	2252
Physical state	Colorless to yellow oily liquid
Solubility	Insoluble in water
Odor	Woody/terpene odor/cloves/ terpene
Density	0.90 g/mL at 20°C
Boiling point	256-259°C at 760 mmHg
Specific gravity	0.899-0.908 at 25°C
Molecular weight	204.35 g/mol
Molecular formula	$C_{15}H_{25}$
XLogP3-AA	4.40
H-Bond Donor	0
H-Bond Acceptor	0
Heavy atom count	15
Formal Charge	0
Rotatable bond count	0
Complexity	293
Isotope atom count	0
Defined atom stereocenter count	2
Undefined atom stereocenter count	0
Defined bond stereocenter count	1
Undefined bond stereocenter count	0
Covalently-bonded unit count	1



Fig. (1). The chemical structure of  $\beta$ -caryophyllene.

In past few years, several methods such as genetic engineering of the terpene synthesizing enzymes in particular up-regulation of terpene synthase 23 (TPS23) and microbial transformation has been shown beneficial in increasing the yield of BCP in naturally occurring plants and animals [62-64]. The plants are known to produce volatile organic compounds which play an important role in determining the quality and nutraceutical properties of fruit and root crops. The excessive production of these volatiles is required in order to improve the nutrceutical properties as well as taste and aroma. Using transcriptome analysis, of genes responsible for synthesis in carrots (*Daucus carota* L.) has been identified which reveal the biotechnological approaches of improved varieties by the manipulation of sesquiterpene synthesizing genes [65].

# **3. PHARMACOKINETICS OF BCP**

The bioavailability and pharmacokinetics of BCP have been investigated in animal studies [66,67]. The lipophilicity (octanolwater partition coefficient) of BCP represented by log P values 6.78 is very high that is indicative of its high penetration across the blood brain barrier and placental transfer [1,68]. The lipophilicity and volatility properties of BCP reduce its bioavailability, Liu *et al.* [67], for the first time demonstrated the pharmacokinetics and bioavailability of BCP (50 mg/kg, orally administered single dose) and BCP in a  $\beta$ -cyclodextrin ( $\beta$ -CD) inclusion complex in rat plasma using analytical technique gas chromatography coupled mass spectrometry with selected ion monitoring mode (GC-MS/SIM). The formulation containing the inclusion complex of BCP with  $\beta$ -CD showed enhanced solubility, dissolution rate, stability and improved bioavailability in rats.

Following oral administration, the peak plasma concentration (Cmax), time to peak plasma concentration (Tmax), mean residence time (MRT), area under the curve (AUC<sub>0-12 h</sub> and AUC<sub>0- $\infty$ </sub>) for free BCP in rat plasma was 0.12  $\pm$  0.02  $\mu g/mL,$  3.50  $\pm$  0.60 h, 4.07  $\pm$ 0.07 h, 7.69  $\pm$  0.13 h, 1.05  $\pm$  0.08 µgh/mL and 1.28  $\pm$  0.10 (µgh/ mL). The Cmax, Tmax, MRT,  $AUC_{0-12h}$  and  $AUC_{0-\infty}$  for BCP/β-CD found  $0.56 \pm 0.35 \ \mu g/mL$ ,  $2.80 \pm 0.80 \ h$ ,  $3.25 \pm 0.65 \ h$ ,  $5.63 \pm$ 0.93 h,  $2.72 \pm 1.22$  µgh/mL and  $2.96 \pm 1.16$  µgh/mL [67]. The BCP/β-CD complex formulation exhibited shorter Tmax and higher Cmax. Also, an increase in the AUC<sub>0-12h</sub> approximately 2.6 times was found than those of only BCP in the plasma. The in vitro dissolution study showed that BCP was quickly released from the formulation [67]. Administration of a single oral dose of BCP is reported to show Tmax greater than 1 h [11]. The favorable bioavailability of BCP in the inclusion complex could be considered a significant success in the direction of its pharmaceutical development, however the human studies for determining pharmacokinetics including metabolism and bioavailability of BCP are yet lacking and need to be investigated for further clinical applications.

# 4. PHARMACOLOGICAL AND MOLECULAR MECHA-NISMS OF BCP

The pharmacological targets of BCP are represented in Fig. (2). Based on its varied properties, BCP could be appropriately considered as a multifunctional and polypharmacological agent for therapeutic development in complex diseases [69]. The therapeutic potential of BCP in different diseases is depicted in Fig. (3). BCP found to regulate the expression and release of various proinflammatory cytokines, chemokines, growth factors, transcription factors, genes, enzymes, adhesion molecules, receptors and heat shock proteins and apoptosis as well as cell cycle associated proteins [6,13,15, 70,71]. Briefly, BCP has been reported to modulate numerous molecular targets in different diseases and disorders by altering gene expression, molecular and cellular signaling pathways or by direct interaction with the targets to thwart the progression and development of numerous disease processes as depicted in Fig. (4). The evidences on pharmacological efficacy and underlying mechanism from different experimental studies demonstrating the therapeutic potential of BCP in numerous diseases are represented in (Table 3).

#### Table 3. The molecular mechanisms of β-Caryophyllene.

• Inhibits intracellular Ca <sup>2+</sup>
• Blocks voltage-dependent Ca <sup>2+</sup> channels
Inhibits pro-inflammatory cytokines
• Inhibits activation of the toll-like receptors
• Inhibits receptor complex CD14/TLR4/MD2
• Activates the mitogen-activated kinases Erk1/2 and p38
Blocks STAT3 signaling cascade
• Enhances phosphorylation of AMP-activated protein kinase (AMPK)
• Enhances phosphorylation of cAMP responsive element-binding protein (CREB)
• Stimulates SIRT1/PGC-1α-dependent mechanism
• Down-regulates anti-apoptotic genes (Bcl-2, Mdm2, Cox2 and Cmyb)
• Upregulates pro-apoptotic genes (Bax, Bak1, Caspase-8, Caspase-9 and ATM)

Pharmacologically, BCP has shown to interact and binds to CB2 receptors [6] and act as a full selective functional agonist in numerous experimental studies including in vitro, in vivo and in silico [11]. The majority of experimental studies aimed to investigate the pharmacological mechanism of CB2 receptors agonists, AM630 (6-Iodopravadoline, CAS 164178-33-0) is used as a pharmacological challenge to demonstrate the CB2 receptor mediated mechanistic activity. AM630 is a ligand, which behaves as a potent and selective inverse agonist or full antagonist on the CB2 receptors (Ki = 32.1 nM at CB2) with 165x selectivity over CB1 receptors, where it showed a weak partial agonism. In computational modeling, BCP was showed to selectively bind with  $\Delta$ 9-tetrahydrocannabinol binding site in the CB2 receptor. It is also remarkable for possessing an unusual cyclobutane ring; a rarity in green chemistry and a unique scaffold that provides an interacting geometry for its binding to CB2 receptors [6]. The CB2 receptors are well characterized members of the G-protein coupled receptors usually bind with G-proteins in the Gi/o family. The activation of the CB2 receptors causes the numerous intracellular effects which may be of cell type and ligand specific and involve the inhibition of various voltage gated Ca2+ channels and adenylate cyclase activity and the activation of K+ channels, resulting in lower levels of cAMP along with activation of MAPK pathways [72]. The CB2 receptor coupled to Gi mediates their cellular effects through inhibition of adenylyl



Fig. (2). The multiple receptor targets of  $\beta$ -caryophyllene.

cyclase and regulation of transcription factors [72]. The activation of CB2 receptors represent an important therapeutic target in numerous diseases [6,15,16].

The cyclobutane pharmacophore of BCP was further utilized as a template for drug discovery and subjected to structural modifications. The modifications generated a series of new monocyclic amides which retained CB2 receptor agonism property in addition to its property in order to inhibit fatty acid amide hydrolase (FAAH), an endocannabinoid degrading enzyme of the endocannabinoid system (ECS) that enhances the tone of endocannabinoid signaling. The generated molecules also elicit the inhibitory properties on endocannabinoid substrate-specific metabolizing enzyme, cyclooxygenase-2 (COX-2) that is an important mediator of inflammatory pathways in the metabolism of arachidonic acid for the generation of prostanglandins [69]. This reveals that the pharmacophore of BCP is amenable to sustain the polypharmacological nature.

BCP has also been found to regulate the nuclear receptors and transcription factor, peroxisome proliferator-activated receptors (PPARs) subtype, PPAR- $\alpha$  and PPAR- $\gamma$ . The activation of PPARs by cannabinoid related molecules elicits numerous advantageous physiological effects and therapeutic benefits [71,73-75]. The PPARs are one of the important members of nuclear receptor superfamily, which function as ligand activated transcription factors and play a critical role in differentiation and proliferation of cells, organogenesis as well as inflammation. They also regulate the expression of hepatic enzymes and participate in glucose homeostasis, insulin sensitivity as well as lipid metabolism [76]. There are three distinct isoforms of PPARs namely PPAR-a, PPAR-y and PPAR-b which show different ligand selectivity and specific distribution in the tissues. Among these different isoforms, PPAR- $\alpha$  is mainly expressed in heart, liver, intestine and macrophages and gets activated by polyunsaturated fatty acids and leukotrienes. Whereas, PPAR-y is mainly expressed in adipocytes but its transcript has also been identified in several other tissues, though in low abundance. PPAR-y plays a vital role in adipocyte differentiation and lipid accumulation as it is activated by polyunsaturated fatty acids and 15d-prostaglandin J2 [77]. Functionally, the PPAR isoforms are similar to those of the steroid receptors and are related to multiple functions started by nutrients, nutraceuticals and phytochemicals. The ligand-binding to cannabinoid receptors increases the activity of mitogen-activated protein kinase (MAPK), which further regulates the activation of PPAR via direct phosphorylation. Though,



Fig. (3). The pharmacological activities and therapeutic potential of  $\beta$ -caryophyllene.



Fig. (4). The biochemical and molecular targets of b-caryophyllene observed in different in vitro and in vivo studies.

the precise mechanisms about cannabinoid and PPARs interactions are unknown, but the PPAR activation by cannabinoid ligands appears partner together to achieve the therapeutic benefits [78]. This provides a basis by which cannabinoids can regulate gene transcription triggered by the dietary interventions. BCP being highly lipophilic has an ability to cross the membranes and reach to the nuclear receptors, thus possess an ability to modulate both the surface and nuclear receptors both.

Further, BCP also found to inhibit the pathways triggered by the initiation of the toll like receptor complex (CD14/TLR4/MD2) and abridged the immune-inflammatory processes in numerous autoimmune diseases [11]. The additional  $\mu$ -opioid receptor activity [79,80] and potent antagonist activity on homomeric nicotinic acetylcholine receptors ( $\alpha$ 7-nAChRs) support its potent antiinflammatory activity. Interestingly, in a study investigated the anxiolytic activity of BCP does not elicit serotonergic, GABAergic and NMDA receptor mediated activities demonstrated by the receptor agonists and antagonists [81].

Numerous studies have demonstrated the in vitro [12,74,82] and in vivo antioxidant mediated protection of various organs [15,16,75,83]. BCP exhibited a potent chain breaking antioxidant and free radical scavenger activity against the highly reactive free radicals such as hydroxyl and superoxide anions [82,84]. BCP has also been found to restore the glutathione redox cycle and effectively inhibit lipid peroxidation, an important pathogenic event and a key culprit in a majority of chronic degenerative and acute organ injuries. BCP has also been found superior than several standard antioxidants such vitamin C and E available for clinical use [82]. BCP (32.5%) isolated from essential oil of Teucrium flavum L. subsp. flavum exhibited free radical scavenging activity and antioxidant potential in DPPH assay [85]. It has also been found to inhibit 5-lipoxygenase, an important enzyme of arachidonic acid pathways that actively participates in the generation of inflammatory mediators and institution of inflammation in numerous inflammatory diseases [13]. The GST inhibitory and lipoxygenase inhibitory activity of BCP was further supported by *in silico* data [86]. The CB2 receptors mediated antioxidant activities were also demonstrated and indicated the additional antioxidant potential of BCP in therapeutic benefits apart from anti-inflammatory activity [82,87]. An interconnected pharmacological properties of BCP mediated by antioxidant and anti-inflammatory properties are represented in Fig. (5).

BCP has been reported to exert strong anti-inflammatory effects in numerous *in vitro* and *in vivo* studies [6,71,79,88,89], which positively contribute to influence several of its biological and therapeutic activities in diseases where low grade and long term inflammation play an important role in etiopathogensis [75,90]. The most documented beneficial effects of BCP are its capacity to regulate pro-inflammatory cytokines and chemokines and prevent the development and progression of immune-inflammatory disorders mediated by CB2 receptors [6]. Various pharmacological activities such as chemopreventive [13], nephroprotective [15], hepatoprotective [16], anticancer [70], ulcerative colitis [71], neuroprotective [75,87,89] and analgesic [91] which in part involve CB2 receptor mediated attenuation of inflammation are depicted in Table **4**.

Several of the studies demonstrate that the anti-inflammatory effects of BCP are mediated by the activation of CB2 receptors. BCP upon binding to CB2 receptors inhibit the enzyme, adenylate cylcase which leads to intracellular Ca<sup>2+</sup> transients and further activates the signaling pathways mediated by Erk1/2 and p38 [11]. Similar to several known CB2 receptor specific ligands, BCP also inhibits the pathways triggered by the activation of the toll-like receptor (TLR) complex; CD14/TLR4/MD2, that lead to the expression of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ ) and stimulates TH1 mediated immune response mediated by CB2 receptor mechanism [6, 97]. Furthermore, BCP showed significant anti-inflammatory effects in various *in vivo* models such as xylene-induced mice ear edema [98], lipopolysaccharides (LPS)-induced inflammation [99] and carrageenan-induced rat paw inflammation [6]. The anti-inflammatory activity of several plants



Fig. (5). Interconnected pharmacological potential of  $\beta$ -caryophyllene.

Table 4. The studies showing CB2 receptors or signaling mediated therapeutic potential of β-Caryophyllene.

Disease	Receptors & Signaling Pathway	References
Ulcerative colitis	CB2 receptors & PPAR-γ pathway	[71]
Chemogenic pain	CB2 receptors & µ-opioid receptors	[80]
Dyslipidemia	CB2 receptors & SIRT1/PGC-1a pathway	[92]
Nephrotoxicity	CB2 receptors	[15]
Inflammation	CB2 receptors & Erk1/2 & JNK1/2 pathway	[6]
Neuropathic pain	CB2 receptors	[80]
Rheumatoid arthritis	CB2 receptors	[6]
Pain	CB2 receptors	[93]
Alcohol addiction	CB2 receptors	[94]
Diabetes	CB2 receptors	[95]
Anxiety / depression	CB2 receptors	[37]
Cerebral ischemia	CB2 receptors	[96]

including *Copaifera multijuga* has been attributed to the presence of high amount of BCP [56]. The essential oil of *Hyptis pectinata* which contain 54.07% of BCP was developed in an inclusion complex with  $\beta$ -cyclodextrin and showed enhanced analgesic and anti-inflammatory pharmacological effects in formalin-induced pain protocol in mice [100]. Due to the multimodal anti-inflammatory mechanisms, BCP appears an important natural agent to treat diseases where immune-inflammatory alterations are the common accompaniment of the pathogenesis of diseases [6].

Additionally, apart from antioxidant and anti-inflammatory activities, BCP showed potent immunomodulatory potential by the inhibition of T-cell immune responses in mouse primary splenocytes [101]. The inhibitory effects of BCP on both, TH1/TH2 cytokines indicate the potential possible benefits of BCP in several autoimmune diseases mediated by CB2 receptors activation and subsequent inhibition of toll like receptors [101]. The CB2 receptors are largely found on the cells of immune origin including spleen and thymus as well as circulating inflammatory cells including T- and B-lymphocytes, natural killer cells, monocytes and neutrophils which suggests an important possible role of BCP in numerous diseases related to modulation of the immune system [102]. As a consequence, the specific activation of CB2 receptors by BCP could offer promising therapeutic applications in numerous autoimmune and immune-inflammatory diseases.

# 5. THERAPEUTIC POTENTIAL OF BCP

# 5.1. BCP in Neurodegenerative Diseases

Oxidative and nitrosative stress, mitochondrial dysfunction, neuroinflammation and subsequent neuronal cell death are the major causes of neuroinflammatory and neurodegenerative diseases [103]. The CB2 receptors have recently been detected in neuronal cells including astrocytes, microglia and oligodendro glial progenitors, suggesting a potential direct regulation of CB2 receptors in brain inflammatory cells following cerebral ischemia [104,105]. Since, CB2 receptor expression is enhanced during the activation of inflammatory cascade, treatment with cannabinoid agents found effective in reducing the inflammation in the early post-ischemic phase [106,107]. The neuroprotective effects of BCP were demonstrated associated with its capacity to down-regulate oxidative stress and pro-inflammatory cytokines [96,108]. BCP was shown to protect neuronal function against damages such as DNA fragmentation, protein oxidation and mitochondrial peroxidation which lead to apoptosis [109]. For instance, BCP isolated from *Salvia fruticosa* has shown protection against  $H_2O_2$ -induced cellular injury in the cultured primary astrocytes of brain [110].

In a recent study, BCP was found to inhibit hypoxia-induced neuroinflammatory processes and cytotoxicity by attenuating generation of reactive oxygen species (ROS) in mitochondria and activation of NF- $\kappa$ B in microglia as well as inhibition of the release of pro-inflammatory cytokines [75]. Likewise, the effects of BCP were shown abrogated by muting the CB2 receptors using RNA interference which demonstrates the CB2 receptor mediated effects [75,89]. Apart from antioxidant and anti-inflammatory effects, additionally high lipophilicity of BCP which indicates its ability to cross blood brain barrier also substantiates its neuroprotective effects [109]. Some of the common neurodegenerative diseases where BCP has been reported effective are described in the next paragraphs.

# 5.1.1. BCP in Alzheimer's and Parkinson Diseases

Alzheimer's disease (AD), a progressive brain disorder involves extracellular accumulation of the beta-amyloid peptide (A $\beta$ ) as amyloid deposits in different regions of the brain, especially in the hippocampus, which interact with various cellular components to trigger signal transduction cascades generating free radicals, and initiating inflammatory response, caspase activation and Ca<sup>2+</sup> deregulation in the brain. AD patients have decreased brain levels of acetylcholine and the inhibition of the acetylcholinesterase (AChE) enzyme favors the accumulation of acetylcholine. Therefore, the potential therapeutic agents for AD are focused on the inhibition of AChE and butyrylcholinesterase (BChE) enzymes inhibitors and BCP has been reported to possess AChE and BChE inhibitory activity and is comparable to standard galantamine [111]. In addition,  $\beta$ -secretase is a trigger enzyme in amyloid  $\beta$  production in the amyloid genic pathway underlying AD. The discovery of a  $\beta$ -secretase inhibitor would be a promising candidate for prevention of AD. The curry leaf, black pepper and turmeric showed both β-secretase and AChE inhibitory activities and can be useful in prevention of dementia [112,113]. BCP isolated from the essential oil of Syzygium aromaticum showed  $\beta$ -secretase inhibitory activity in addition to AChE inhibition. Considering the B-secretase inhibition activity as well as AChE inhibition, BCP could be a promising candidate for AD [113].

Sharon Anavi Goeffer and colleagues patented (US Patent No. US20150051299 A1), a composition comprising BCP and a pharmaceutically effective carrier for use in treating schizophrenia in the mouse model of schizophrenia and psychotic effects in humans induced by phencyclidine (PCP), an NMDA antagonist [114]. PCP induces long-lasting schizophrenic-like effects in mice that lasted into adulthood. BCP treatment reversed the effect of PCP on prepulse inhibition and reduced the level of stress and anxiety following the reversal of the action of PCP on locomotor behavior. BCP also reversed the effects of BCP such as ambulation and rearing behaviors by AM630 demonstrate CB2 receptor mediated mechanisms. The study showed that BCP similar to other CB2 agonists may be useful in treating schizophrenia.

In recent years, for the treatment of AD, ECS in particular activating CB2 receptors has emerged as a potential therapeutic approach [115]. Subsequently, in experimental studies, PPAR- $\gamma$  agonists have shown to protect against inflammation, reduce amyloid plaque burden and reverse the disease-related behavioral impairment in AD [116]. The  $\alpha$ 7-nAChR antagonism has also showed therapeutic potential against AD [117]. Nonetheless, King *et al.* [118] have recently shown direct coupling between G-proteins and

nAChRs in neurons that can be reasonably speculated to be associated with the multiple pharmacological action of BCP. Recently, Siddique *et al.* [119] have found that *Centella asiatica* leaf extract which contains BCP as a major constituent improved behavioral abnormalities, reduced oxidative stress and apoptosis in the brains of drosophila model of Parkinson's diseases [119]. Considering the CB2 activation and PPAR- $\gamma$  pathways [113] and  $\alpha$ -7 nAChR antagonist properties of BCP, it appears that BCP may be a useful drug in neurodegenerative diseases; however further studies are yet to be undertaken.

# 5.1.2. BCP in Epilepsy

Epilepsy is one of the chronic brain diseases characterized by frequent seizures, which affects more than 50 million people worldwide. Since the pharmacotherapy of epilepsy often involves long term treatment with antiepileptic drugs (AEDs), however still the drugs are not able to satisfactorily control the seizures and drugresistant epilepsy constitutes a real therapeutic challenge in epilepsy management. The current efforts are aimed to inhibit epileptogenesis; a process of transforming a normal brain into an epileptic brain. Recently, CB signaling mechanisms have revealed that the CB agonists may modulate the hyperexcitability phenomena by targeting CB receptors involving the downstream effectors such as nitrergic dependent cGMP pathway [120].

Recently, BCP have reported to decrease seizure activity, mortality and inhibit lipid peroxidation as well as improve the activity of antioxidant enzymes [121]. It also significantly reduced cerebral pro-inflammatory cytokines. The CB2 receptors are known for their inhibitory activity in neuroinflammation and oxidative stress and BCP has been shown to exhibit neuroinflammatory cascade, however the role of CB2 receptor mediated antiepileptic activity of BCP yet to be determined.

# 5.1.3. BCP in Cerebral Ischemia

Ischemic injury to the cerebral tissues elicits an intricate biochemical changes that eventually lead to neuronal cell death either from apoptosis or necrosis [122]. Currently, the attention has been shifted from CB1 to CB2 receptors which are mostly expressed in immune cells including brain resident microglial cells as modulating CB2 receptors do not cause unwanted neuropsychiatric adverse effects. The CB2 receptors played an important role in modulation of inflammation (altering production of the pro-inflamatory cytokines and expression of COX-2) and iNOS expression [122]. The CB2 receptors have been largely involved in the anti-inflammatory and iNOS expression modulatory effects of different cannabinoids [123,124]. Selective CB2 receptor agonists found to diminish the post-stroke enhancement of leukocyte/endothelial cells interaction, adhesion molecule expression and disruption of blood-brain barrier in animal models of middle cerebral artery occlusion and focal cerebral ischemia [96,122]. They have been shown to regulate postinjury microglial activation and inflammatory functions by reducing activation of microglia and down regulating expression of inflammatory genes such as IL-6, TNF-α, MCP-1 and MIP-1α [125,126].

The protective effect of BCP in cerebral ischemia has been studied in the rat cortical neurons/glia mixed cell cultures and in the rat model [96]. BCP found to lessen neuronal injury and the depolarization of mitochondrial membrane instigated by oxygen-glucose deprivation/re-oxygenation in the cortical cells and improved phosphorylation of AMP-activated protein kinase (AMPK) as well as cAMP responsive element-binding protein (CREB) and expression of the CREB target gene product, brain-derived neurotrophic factor (BDNF). In rats following cerebral ischemia, BCP treatment reduced the cerebral infarct size and edema and up-regulated the expression of phosphorylated CREB and BDNF in the neurons. Contrariwise, the abrogation of the beneficial effects of BCP by selective CB2 receptor antagonist, AM630 demonstrates the mediation of cortical CB2 receptors. Additionally, the attenuation of the neuroprotective effects of BCP by selective inhibitors of AMPK and CREB clearly demonstrates that the activation of cortical CB2 receptors by BCP profoundly attenuates cerebral ischemic injury, potentially through modulation of AMPK/CREB signaling.

In another study, Chang *et al.* [108] demonstrated the protective properties of BCP in rat model of stroke induced by occlusion of middle cerebral artery and consequent reperfusion. BCP treatment reduced infarct size, improved neurologic deficits and attenuated the pro-inflammatory cytokines. The findings suggest that cortical CB2 receptors represent a putative therapeutic target for cerebral ischemia and BCP by activating cortical CB2 receptors appear a promising agent for neuroprotection in cerebral ischemia.

# 5.1.4. BCP in Depression and Anxiety

Recent anatomical, functional, genetic and pharmacological studies indicate that the ECS, particularly CB2 receptors are notably expressed in several regions of the brain that closely participate in the regulation of anxiety and depression disorders [127]. For the first time, Galdino and colleagues [81] demonstrated the antianxiety property of BCP and the possible underlying mechanisms of action explicitly by investigating the role of GABAA/BZD or 5-HT1A receptors using flumazenil, an antagonist of GABAA receptors and NAN-190, an antagonist of 5-HT1A receptors. The results demonstrate that the antagonize the anxiolytic effects. The findings revealed that the antagonize the anxiolytic effects. The findings revealed that the anxiolytic effects of BCP are not mediated by benzodiazepine or serotonergic mechanisms and this could be related to its CB2 receptor activating property [81].

Since, BCP has been shown to possess CB2 receptor activating property and this property has been showed to confer protection against different diseases, we have recently investigated the effects of BCP in experimental models of anxiety and depression in mice using a battery of tests. The evaluation of anxiety was performed using open field test, elevated plus maze and marble burying tests and parameters for the depression were evaluated by using forced swim test, novelty-suppressed feeding test and tail suspension tests [128]. BCP treatment found to ameliorate all the studied parameters of anxiety and depression and demonstrate the anti-compulsive like effect. The CB2 receptor dependent mechanism was demonstrated by the pretreatment with AM630, a CB2 selective receptor antagonist which abolished the beneficial effects of BCP on anxiety and depression.

From available studies, BCP appears to possess antidepressant and anxiolytic activity mediated by its inherent CB2 receptor dependent mechanisms rather involving other receptors such as GABAergic, NMDA, serotonergic and glutaminergic [81]. The targeting of CB2 receptors by selective agonists may provide alternative therapeutic agents for the pharmacotherapy of mood disorders and offer exciting prospects for the therapeutic potential of BCP.

# 5.1.5. BCP in Alcohol Addiction

Alcohol dependence is a grave social, medical and economical issue due to its concerns of morbidity and mortality throughout the world. Since the advent of ECS, accruing evidences demonstrate that the alcohol interacts with the molecular components of ECS and play a significant and pervasive role in the etiology of alcohol addiction including the rewarding and reinforcing effects of alcohol. The findings suggest that modulation of the CB2 receptors among the components of ECS could be an important and key therapeutic target for alcohol dependence as well as drug addiction [129,130]. Several recent studies using CB2 knock outs and pharmacological models of alcohol addiction, suggested that brain CB2 receptors play an important role in consumption, dependence, reward and reinforcement of alcohol and the targeting of CB2 receptors could be an important therapeutic strategy [129].

Recently, BCP has been found to decrease alcohol consumption and preference following two-bottle choice method containing alcohol and water in a dose-dependent manner [94]. BCP also inhibited ethanol-induced conditioned place preference acquisition in alcohol-induced conditioned place preference and exacerbated duration of the loss of righting-reflex in mice. Additionally, BCP was found not to affect the total fluid intake and appear advantageous over other agents used for alcohol addiction.

BCP was found to decrease alcohol consumption and preference in a dose-dependent manner [94]. BCP also did not alter taste evidenced by no difference with intake of graded concentrations of saccharin or quinine and did not alter body weight during the treatment period. All these beneficial properties make BCP an ideal agent for its potential indication in alcohol addiction. The abrogation of the beneficial effects of BCP by AM630, a selective CB2 receptor antagonist clearly demonstrates the anti-addictive property of BCP in alcohol addiction. The findings revealed that CB2 receptors participate in alcohol dependence and sensitivity, therefore agents activating CB2 receptors like BCP may be a potential agent of natural origin for the pharmacotherapy of alcohol addiction and dependence [94].

# 5.2. BCP in Pain and Inflammation

Inflammation is a complex biological response that occurs when the body is exposed to infective agents or to physical or chemical changes [131,132]. Several essential oils or essential oils containing sesquiterpenes which are used in aromatherapy are believed to exert beneficial effects due to their anti-inflammatory activity [132]. The efficacy of BCP alone and essential oils containing BCP as a major active constituent has been investigated in numerous experimental studies [131-134]. In the modulation of acute and chronic pain, the ECS has been shown to play a significant role and appear an important therapeutic target [133,134]. Both, CB1 and CB2 receptors have been shown to participate in pain and inflammation. However, the absence of CNS side effects put forward CB2 receptor activation seem an attractive target for the treatment of pain as demonstrated in well-validated models of acute pain, persistent inflammatory pain, post-operative pain, cancer pain and neuropathic pain [135-137]. BCP appears effective in nociception, persistent inflammatory pain, chemogenic pain and neuropathic pain mediated by CB2 receptor activation [6,79,80,93].

# 5.2.1. Neuropathic Pain

Neuropathic pain is caused by disease or injury of the nervous system and includes various chronic conditions that affect up to 8% of the population including both type-1 and type-2 diabetes and affect over 90% of the diabetic patients [138]. It involves aberrant ectopic activity in nociceptive nerves, peripheral and central sensitization, impaired inhibitory modulation, and pathological activation of microglia [139]. The treatment of neuropathic pain provides only symptomatic relief and may include non-pharmacological, pharmacological, and interventional therapies. Most extensive evidence is available for pharmacological treatment and currently recommended first-line treatments including antidepressants (tricyclic agents and serotonin-norepinephrine reuptake inhibitors) and anticonvulsants (gabapentin and pregabalin) and individualized physical and psychological therapies.

The role of cannabinoids in neuropathic pain is well-explored in mouse models of chronic pain, including peripheral neuropathy (chronic constriction nerve injury, CCI), tonic inflammatory pain (the formalin test) and short and long-term inflammatory pain (complete Freund's adjuvant, CFA and carrageenan tests) in knockout and wild-type mice [138]. Till date, the most promising therapeutic action of CB2 receptor agonists is in neuropathic pain [135-137]. Correspondingly, BCP has shown substantial maximal efficacy in neuropathic pain and found superior over synthetic CB2 ligands and appear beneficial as adjuvant with opioid analgesics and other drugs [79, 80, 93].

Klauke et al. [93] showed that BCP reduced inflammatory (late phase) pain responses in the formalin test in a CB2 receptor -

dependent manner in a neuropathic pain model. BCP treatment ameliorated thermal hyperalgesia and mechanical allodynia and decreased spinal neuro-inflammation. There were no signs of tolerance to the anti-hyperalgesic effects of BCP even after chronic treatment. Paula-Freire *et al.* [79] showed that BCP treatment significantly minimized the pain evaluated in murine models of acute induced by hot plate test (thermal nociception) and the formalin test (inflammatory pain) and chronic pain induced by chronic constriction injury of the sciatic nerve induced hyper-nociception was measured by the hot plate and von Frey tests. Further, the role of opioid and ECS was demonstrated by the reversal of the nociceptive effect by naloxone and AM630, indicating the participation of both the opioid and ECS.

The effects of peripheral cannabinoid and opioid systems in the antinociception produced by intraplantar injection of BCP were substantiated by Katsuyama *et al.* [80] in a mice model of capsaicin-induced nociception BCP dose-dependently attenuated capsaicin-induced nociceptive response mediated by CB2 receptors and  $\mu$ -opioid receptors as confirmed by pharmacological challenge studies. The activation of CB2 receptors by BCP stimulates the release of  $\beta$ -Endorphin and inhibits proinflammatory cytokines and confers antinociception. Also, morphine-induced antinociception was increased even by a low dose of BCP which indicated that the use of BCP as an adjunct to synergistically improve the efficacy and circumvent the undesirable side effects of opioids by reducing its dose [80].

Further, in another study the combination of BCP and docosahexaenoic acid (DHA) showed modulation of inflammatory pain responses in synergistic ways and do not interact with each other [91]. DHA is found in salmon and seaweed has well-known beneficial activities which enzymatically converted into bioactive autacoids, possessing inflammation-resolving properties. The *in vitro* acute toxicity of BCP and DHA alone and in combination was determined in mouse immortalized NIH3T3 fibroblasts and human U373-MG astrocytes (derived from a human astrocytoma line) and found safe at lower concentrations. The administration of BCP or BCP plus DHA reduces pain behavior in a model of persistent pain, the formalin test and did not affect gonadal hormones, testosterone and estradiol.

Based on the available studies demonstrating the dual targeting of CB2 receptors and opioid receptors, and synergistic action of BCP with other agents, the combination appears a promising candidate for the treatment of chronic pain due to high potency and comparable safety and efficacy along with low adverse effects profiles [79,80, 91].

# 5.2.2. BCP in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that causes deformity of the joints and physical disability. Current treatment modalities for rheumatoid arthritis either produce symptomatic relief (NSAIDs) or modify the disease process (DMARDs). Though effective, their use is also limited by their side effects. As a result, the interest in alternative, well-tolerated anti-inflammatory remedies has re-emerged. Numerous studies have shown that ECS especially CB2 receptor has an important role in the pathophysiology of RA [140, 141]. The agents acting on molecular components of ECS are reported to inhibit synovial inflammation, hyperplasia, and cartilage destruction in RA [140-142].

In particular, specific activation of CB2 may relieve RA by inhibiting not only the production of autoantibodies, proinflammatory cytokines, and MMPs, but also bone erosion, immune response mediated by T cells and the proliferation of fibroblasts like synoviocytes [143]. Several selective CB2 receptor agonists have demonstrated analgesic activity across multiple preclinical pain models and showed that CB2 receptor activation confer therapeutic benefits in arthritis by reducing the pain and modulating the immune-inflammatory changes [142]. For the first time, Gertsch *et*  *al.* [144] demonstrated the anti-inflammatory effect of BCP in carrageenan-induced inflammation mediated by CB2 receptor dependent mechanisms. Numerous studies are available wherein BCP alone or plant extracts containing BCP have been revealed effective in treatment of RA [6,97,145,146]. The effects of BCP isolated from *Cordia verbenacea* on acute inflammatory responses elicited by LPS were studied in acute inflammation model in rat paw [99].

In another study, BCP treatment showed effective in reducing platelet activating factor-, bradykinin- and ovalbumin-induced mouse and rat carrageenan-induced paw edema. Furthermore, it reduced the production of TNF- $\alpha$ , PGE2, iNOS and COX-2 expression and the effects were comparable to the standards drug, dexamethasone [146]. BCP showed to effectively reduce the neutrophil migration and activation of NF-kB induced by LPS in the rat paw. However, failed to interfere with activation of the MAP kinases, ERK, p38 and JNK and inhibit LPS-induced NF-kB activation and neutrophil migration [146].

In osteoarthritis (OA), a recent study has evaluated that BCP isolated from the essential oil of Eryngium duriaei subsp. juresianum in primary human chondrocyte cultures stimulated with IL- $1\beta$  as an in vitro cartilage degradation model that represents the damage observed in OA [88]. Though, BCP was found inactive in inhibiting IL-18-induced NO production. OA is a multifactorial degenerative joint disease characterized by inflammation and progressive loss of the articular cartilage, associated with changes in the subchondral bone and other joint tissues. It is one of the most frequent chronic diseases and progressive that leads to functional decline and loss in quality of life, with important health care and society costs. However, the observation of no efficacies of BCP in OA was contrary to the therapeutic benefits of BCP in RA in several studies. Though, the data was from an in vitro study, however previous evidences are from animal models of arthritis demonstrate the anti-inflammatory effects by activating cannabinoid CB2 receptors [6,71,99]. Thus, further studies are needed to translate the therapeutic effects observed in animal studies.

# 5.2.3. BCP in Spasmodic Pain

The role of BCP in spasmodic pain mediated by acetylcholine and histamine has been demonstrated measuring the contractility of the guinea-pig ileum [147]. BCP isolated from the essential oil of *Plectranthus barbatus* (Indian Coleus) inhibited the phasic contractions evoked by acetylcholine with no effect on histamine- or barium chloride-induced contractions. The spasmolytic and intestinal relaxant activity of BCP depends on exchange of  $Ca^{2+}$  from internal stores and found independent of antagonism of receptors for neurotransmitters or autacoids [147].

In another study, the effects of BCP isolated from the essential oil of *Pterodon polygalaeflorus* were evaluated on ileum smooth muscle [148]. BCP inhibited the contractions induced by acetylcholine and KCl and relaxed the basal tone of ileum smooth muscle in a concentration-dependent manner. The effects exerted by BCP were not unchanged by hexamethonium, L-nitroarginine methyl ester or indomethacin. Whereas, BCP reduced calcium chloride-induced contractions in the ileal preparations pretreated with acetycholine under Ca<sup>2+</sup> free condition and in the presence of verapamil, a calcium channel blocker. In the presence of high KCl and Ca<sup>2+</sup> free conditions, BCP decreased the contractions induced by barium, similar to verapamil. The myorelaxant and antispasmodic effects of BCP mediated through an intracellular mechanism and the inhibitory effect on intestinal contractility appear myogenic [148].

# 5.3. Local Anesthetic Effect of BCP

The local anesthetic activity of BCP isolated from *Syzygium aromaticum* (clove) was demonstrated using conjunctiva reflex test in rabbits (*in vivo*) and rat phrenic nerve hemi-diaphragm (*in vitro*) [5]. BCP found to dose dependently reduce the contractions in-

duced electrically in rat phrenic hemi-diaphragm. The authors suggest that BCP hold the local anesthetic activity owing to the distinctive structure and pharmacophore. The structural derivatives and motifs need to be investigated further for the possible therapeutic application as a local anesthetic.

# 5.4. BCP in Ageing and Longevity

Aging is a time dependent devastating process accompanied with numerous chronic disorders like neurodegenerative, cardiovascular, metabolic diseases and cancer causing a burden to the society [149]. Pant *et al.* [150] in a study using the well-established *Caenorhabditis elegans* model system elucidated the anti-stress and longevity promoting action of BCP. It prolonged the lifespan, reduced oxidative stress and improved cellular redox homeostasis in *C. elegans.* It also modulated feeding behavior, pharyngeal pumping and body size and reduced the intestinal lipofuscin levels. Further, using molecular docking the authors predicted the potential molecular targets of BCP and found its interaction with several ageing related genes such as SKN-1, SIR-2.1 and DAF-16 [150].

The docking was further supported by *in vivo* studies which showed increased lifespan of mev-1 and daf-16 employing the mutants and transgenic strains revealing underlying genetic basis of mechanism. BCP found to regulate several genes involved in the antioxidant defense and oxidative stress, xenobiotic detoxification, ageing and longevity. The studies demonstrate that BCP is a promising agent for ageing and longevity and potential as an epigenetic modifier by regulating multiple signaling pathways.

# 5.5. BCP in Gastrointestinal Disorders

Inflammatory bowel disease (IBD) is an incurable disease which affects millions of people in industrialized countries. Anecdotal and scientific evidence suggests that Cannabis use may have a positive impact on IBD patients [151]. The activation of cannabinoid receptors by endocannabinoids impacts on a number of gastrointestinal functions including IBD [152]. There are several reports on the protective effect of CB2 receptors agonists in gastrointestinal tract [153,154]. Likewise, the role of BCP in gastrointestinal disorders such as abdominal pain, IBD and peptic ulcer has been evaluated in the *in vitro* and *in vivo* animal models.

# 5.5.1. BCP in Ulcerative Colitis

In gastrointestinal disorders, IBD is a group of chronic disorders comprising Crohn's disease (CD) and ulcerative colitis (UC). Their etiologies are not well-understood but they are characterized by an imbalanced production of pro-inflammatory mediators, enzymes and the formation of ROS as well as increased recruitment of leukocytes to the site of inflammation. IBD is a chronic intestinal inflammation caused by hyper activated effector immune cells that produce pro-inflammatory cytokines [155]. Recent studies have shown that the CB2 receptor plays a critical role in mediating protection against intestinal inflammation and CB2 receptor agonists may serve as a therapeutic modality against IBD [153,154].

The role of CB2 receptors in well-known innate immunity has shown expressed in immune cells, such as macrophages, CD4+, and CD8+ T cells, monocyte and polymorphonuclear neutrophils, involved in the IBD [154]. The effects of BCP were evaluated in dextran sulfate sodium and oxazolone-induced mice models of ulcerative colitis [156]. BCP treatment has been found to suppress the shortening of colon length, and offset the loss of body weight and dose dependently improved disease activity index and healthy appearance and recovered weight loss. Further, BCP caused significant reduction in the colonic macro- and microscopic damage, myeloperoxidase and N-acetyl glucosaminidase activities and levels and mRNA expression of colonic TNF- $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$  and keratinocyte-derived chemokine [71]. BCP showed reduction in inflammation by modulating signaling pathway and inhibiting activation of extracellular signal-regulated kinase 1/2, NF- $\kappa$ B, I $\kappa$ B-kinase  $\alpha/\beta$ , cAMP response element binding and the expression of caspase-3

and Ki-67. Moreover, BCP enhanced IL-4 levels and fork head box P3 mRNA expression in the mouse colon and reduced cytokine levels (TNF- $\alpha$ , keratinocyte-derived chemokine, and macrophageinflammatory protein-2) in a culture of macrophages stimulated with lipopolysaccharide [71]. BCP has also been shown to activate the PPAR- $\gamma$  and participate in the anti-inflammatory effects. Pretreatment with GW9662, a PPAR-y antagonist GW9662 and AM630, a CB2 receptor agonist significantly reversed the protective effect of BCP and clearly demonstrates the mechanism of protection [71]. Recently, PPAR- $\gamma$  agonists have shown to effectively control the inflammatory processes of the gastrointestinal tract and exert beneficial effects on macroscopic and histopathological features of colitis in numerous studies [157, 158]. The CB2 receptor activation by BCP has shown to trigger the PPAR-y pathway and improve colitis and suggest BCP as a possible therapy for the treatment of IBD.

A recent study developed coated tablets of CIN-102 containing a mixture of trans-cinnamaldehyde, trans-2-methoxycinnamaldehyde, cinnamyl acetate, linalool, cineol, benzyl benzoate and BCP for colitis, in particular for IBD induced in mice by dextran sodium sulfate [159]. The volatile and lipophilic liquid state of the formulation was a challenge in delivery of CIN-102, thus pellet cores were developed in a way to optimize the release of drug only in the colon to achieve maximum for therapeutic concentration at the site of action. The coated pellets and mini-tablets showed release *in vitro* and appear effective *in vivo* by lessening load of luminal and mucosal enterobacteria along with improved disease activity and clinical course of the intestinal inflammation. The efficacy of these newly coated pellets and matrix mini-tablets showed promise for future development of CIN-102 as a controlled release synergistic antibacterial preparation for colitis.

# 5.5.2. BCP in Peptic Ulcer

Gastric or peptic ulcer constitutes major complaint which affects human gastrointestinal tract and presents major global health problem both in terms of morbidity and mortality. Consumption of alcohol, pain killers and several other drugs has been identified as a major risk factor responsible for acute gastric mucosal injury in humans and presents life-threatening hemorrhages that require immediate medical intervention. The inhibition of gastric acid secretion and enhancing the mucosal defense has been proven to be a powerful therapeutic approach in the treatment of gastric and duodenal ulcer [160].

The gastric cytoprotective effect of BCP was investigated in rats by Tambe *et al.* [161] by significantly inhibiting gastric mucosal injuries induced by necrotizing agents, although it failed to prevent water immersion stress- and indomethacin-induced gastric lesions [161]. Though, BCP did not affect the secretion of gastric acid and pepsin but found devoid of gastric mucosal damage a typical adverse effect of non-steroidal anti-inflammatory drugs. BCP manifested cytoprotective effects conferred a dual/two-dimensional efficacy, gastric protectants as well as anti-inflammatory and devoid of gastric adverse effect.

#### 5.6. BCP in Diabetes

The incidence of type 2 diabetes mellitus (T2DM) increases dramatically worldwide and has created an enormous health care burden [2]. Obesity, dyslipidemia and insulin resistance are major risk factors for the development of TDM but the major factor leading to the disease is failure of the insulin-producing beta cell mass to compensate for increasing insulin demands of the body. Insulin resistance is critical in the development and progression of diabetes mellitus and its complications. By decreasing oxidative stress and whereby decreasing insulin resistance, it may be possible to decrease complications of diabetes mellitus. Thiazolidinediones (TZDs) are clinical insulin-sensitizers acting through a canonical peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ )dependent insulin trans-activation pathway. The PPAR- $\gamma$  agonist, such as pioglitazone and rosiglitazone are successfully used for the treatment of T2DM and insulin resistance despite several challenges and pitfalls [162].

In several studies, BCP has been shown to up-regulate PPAR- $\alpha$ and - $\gamma$  and may regulate glucose and lipid metabolism [16,73,75,92]. PPAR- $\gamma$  also participates into the programmed differentiation of adipocytes by enhancing the AMPK activity, a master energy sensor which regulates diverse metabolic pathways, increases mitochondrial activity and biogenesis in muscles and is responsible for the inhibition of adipogenesis [163,164]. The activation of mouse  $\beta$ -cell CB1 and CB2 receptors has been shown to decrease cyclic AMP, increase calcium and potentiate glucosestimulated insulin secretion stimulate insulin secretion to maintain glucose homeostasis [165].

Recently, Suijun *et al.* [95] have investigated the effects of BCP on glucose stimulated insulin secretion (GSIC) and explored its underlying mechanisms. Glucose-stimulated insulin secretion plays an important role in the control of metabolic fuel homeostasis and its impairment is a key factor for the pathogenesis in T2DM. The administration of BCP dose dependently improved glucose stimulated insulin secretion in MIN6 cells [95]. Activation of CB2 receptors present in  $\alpha$ - and  $\beta$ -cells including MIN6 cells has shown to modulate glucose stimulated insulin secretions by activation of small G-proteins e.g., Arf6, Cdc42, and Rac1, which play a critical role in controlling signaling events participate in regulating trafficking of insulin-laden secretory granules to plasma membranes for docking and fusion.

BCP effects on glucose-stimulated insulin secretion have shown CB2 receptor dependent as inhibition of CB2 receptors by a specific inhibitor or specific RNA interference abolished the effects of BCP. Further, BCP treatment leads activation of small G-proteins and silencing of G protein, Arf6 abolished the effects of BCP on glucose stimulated insulin secretion. BCP appears to regulate glucose stimulated insulin secretion in pancreatic  $\beta$ -cells and Arf6 has been shown to mediate the effects of BCP, a novel mechanism in glucose homeostasis mediated by CB2 receptors.

Additionally, the antihyperglycemic effects of BCP by modulating glucose homeostasis in streptozotocin-induced diabetic rats were demonstrated [166]. Oral administration of BCP decreased glucose with increased plasma insulin levels and ameliorated the altered activities of carbohydrate metabolic enzymes to near normal in liver, kidney and skeletal muscles in a dose dependent manner. The effects were comparable to standard hypoglycemic drug; glibenclamide and the insulinotropic effect of BCP were further supported by immunohistochemical studies.

BCP present in the powdered dried leaves of *Eugenia punicifolia* found effective for the treatment of type-2 diabetes in a pilot, non-controlled study recruited fifteen patients who received extract for 3 months. A significant decrease in glycosylated hemoglobin, basal insulin, thyroid-stimulating hormone, C-reactive protein, and both systolic and diastolic blood pressure was translated into therapeutic outcome and concluded promising as an adjuvant in the treatment of type-2 DM [2]. Taken all together, the stimulation of AMPK and CB2 receptor activation and consequent activation of PPAR- $\gamma$  lead to the regulation of genes implicated in lipid metabolism and adipocytes differentiation, eventually decreasing adiposity and insulin resistance. The absence of typical adverse effects of PPAR- $\gamma$  such as weight gain and edema with BCP is encouraging because of its further use for the treatment of diabetes and its complications.

# 5.7. BCP in Hypertriglyceridemia

Hypertriglyceridemia is considered as an independent risk factor for atherosclerosis and coronary artery disease, the leading causes of morbidity and mortality worldwide. The defective clearance of plasma triglyceride-rich lipoproteins is considered one of the major players in the pathogenesis. The PPAR- $\alpha/\gamma$  agonist activity has been demonstrated to be one of the mechanisms of BCP [92,95]. Considering the linkage between CB2 receptors and PPAR- $\gamma$  and their role in adipogenesis and lipotoxicity, BCP has been shown to regulate fatty acid oxidation through a signaling/transcriptional mechanism, the PGC-1 $\alpha$  pathway [92]. The CB2 and PPARs interaction represents an important therapeutic target for inflammatory disorders including neurodegenerative and metabolic diseases. BCP-induced activation of CB2 receptor triggers the activation of PPAR- $\alpha$  signaling exerting several physiological and therapeutic effects in insulin resistance [73]. The PPAR- $\alpha$  agonist property of BCP expands its therapeutic spectrum in regulating hepatic lipid metabolism including triglycerides accumulation, fatty acid uptake, and oxidation.

Very recently, Wu et al. [73] have shown that BCP induced hepatic fatty acid uptake and oxidation lipid loaded in HepG2 cells. BCP treatment also increased the expression of the genes involved in fatty acid uptake and β-oxidation and suppressed the LXR-SREBP-1c pathway, which led to an increase in fatty acid uptake, oxidation and a reduction in intracellular triglyceride concentrations in hepatic lipid metabolism. BCP induces the expression of PPAR-a and its target genes in hepatocytes, which induces the catabolism of fatty acids by increasing the gene expression of the rate-limiting enzymes involved in fatty acid uptake and fatty acid oxidation, including fatty acid transport protein 4 (FATP4), acyl-CoA synthetase (ACS), carnitine palmitoyltransferase (CPT1) and acyl-CoA oxidase (ACOX). The FATP4 is the main protein involved in fatty acid uptake, which can facilitate the uptake of long chain fatty acids in the liver. The up-regulation of ACS prevents an efflux of fatty acids from the cell through esterifying fatty acids to acyl-CoA derivatives. Both, CPT1 and ACOX enzymes are the rate-limiting enzymes involved in fatty acid target genes of SREBP-1c, fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD1). CPT1 is responsible for the transport of fatty acids into the mitochondria; and ACOX is responsible for the oxidation of acyl-CoA esters to reduce fatty acid levels.

Additionally, the activation of PPAR- $\alpha$  by BCP has been shown to suppress the LXR-SREBP-1c pathway through reducing the binding of LXR/RXR to LXRE and can regulate the gene expression of SREBP1c and SCD1, which is required for the synthesis of triglycerides, cholesterol esters and phospholipids oxidation. PPAR $\alpha$  is primarily expressed in heart, liver, macrophages and intestines, and is activated by polyunsaturated fatty acids and leukotriene B4. Recently, several studies have demonstrated the activation/ interaction of PPARs by cannabinoids or related molecules and several pathways have been suggested to be involved including a direct binding of cannabinoids to PPARs, or the conversion of cannabinoids into metabolites that in turn activate PPARs or the interaction of cannabinoids with cell surface receptors, initiating intracellular signaling cascades that lead to the activation of PPARs [104,167,168].

The CB2 and PPARs mediated mechanism of BCP offer novel therapeutic possibilities to treat metabolic diseases and lipid dysregulation, altogether. The polypharmacological and multifunctional properties make BCP a promising molecule with pleiotropic effects and numerous therapeutic applications for hypertriglyceridemia, insulin resistance and metabolic syndrome. However, further direct evidences from systematic studies are required to demonstrate the therapeutic effects of BCP in metabolic syndrome, hypercholesterolemia and insulin resistance.

#### 5.8. BCP in Endometriosis and Dysmenorrhea

The herbal treatments are being popularly used for the women's conditions such as uterine fibroids (benign tumors of uterine smooth muscle); menorrhagia (excessive uterine bleeding); endometriosis (growth of endometrial tissue outside of the uterus); and hot flashes (sudden brief sensations of heat commonly experienced during menopause) [169]. Among them, endometriosis is a common mysterious and fascinating gynecological condition with diverse clinical manifestations, highly variable and unpredictable clinical course with decreased quality of life. Endometriosis is being recognized as a condition in which ectopic endometrial cells exhibit abnormal proliferative and apoptotic regulation in response to appropriate stimuli [170]. Diverse signaling pathways have been studied in endometriosis that correlates with the abnormal proliferation or growth of the endometrial cells.

The ECS is indeed involved in the regulation of these pathways and cannabinoids are endowed with antiproliferative and antifibrotic properties, in addition to their psychogenic and analgesic effects [171,172]. The role of cannabinoids mediated by CB1 and CB2 receptors which are expressed in epithelial and stromal cells derived from the endometrium and by deep-infiltrating endometriosis nodules in the proliferation of endometriosis cells has been demonstrated. Simultaneously, experimental evidence is accumulating to suggest that medicinal botanicals have anti-inflammatory and pain-alleviating properties and hold promise for the treatment of endometriosis [173].

The anti-inflammatory and analgesic activity of BCP has been well studied in several pain and inflammation models. The selective cannabinoid receptor agonists, such as WIN 55212-2, appear to have a favorable action in limiting cell proliferation and in controlling pain symptoms in deep infiltrating endometriosis which is characterized by chronic pain, hyperproliferation of endometriotic cells and fibrosis [174]. Conversely, endometrial cell migration tends to be stimulated by the cannabinoid agonists,  $\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC) and N-arachidonyl glycine which are full agonists at GPR18 receptors [175, 176]. Abbas et al. in 2013 [177] showed the effect of BCP on endometriosis and fertility by suppressing the growth of endometriotic implants by 52.5% in adult female rats. It also induced apoptosis in luminal epithelium of the cyst and endothelial cells of blood vessels. BCP-treated rat cysts revealed the presence of active mast cells and eosinophils in ultrastructural studies.

Recently, another study in humans has indicated the BCP rich blended essential oil consists of *Lavandula officinalis* (lavender), *Salvia sclarea* (clary sage) and *Origanum majorana* (marjoram) in a 2: 1: 1 ratio, diluted in cream at 3% concentration applied topically on their lower abdomen from the end of the last menstruation continuing to the beginning of the next menstruation in the patients of the dysmenorrhea [178]. Dysmenorrhea refers to the occurrence of painful menstrual cramps of uterine origin and is a common gynecological condition with considerable morbidity. The formulation showed relief in primary dysmenorrhea and reduced the duration of menstrual pain. Considering the pain relieving and antiinflammatory property BCP could be beneficial in the management of primary dysmenorrhea.

Additionally, it appears safer over non-steroidal antiinflammatory drugs which are commonly used in dysmenorrhea. The behavioral approach for the treatment of dysmenorrhea may include both physical and cognitive procedures and focus on both physical and psychological coping strategies for dysmenorrhea symptoms rather than modification of any underlying organic pathology. Based on the positive findings, BCP represents a promising novel and non-toxic therapeutic option for patients with endometriosis. However, the CB2 mediated therapeutic effect and mechanism yet to be investigated.

# 5.9. BCP in Cancer

The sesquiterpene compounds represent a large and diverse group of biologically active plant compounds that possess antiinflammatory [108] and chemopreventive and antitumor activity [179-181]. The antitumor activity related to cell-cycle arrest, differentiation, apoptosis induction through the intrinsic pathway, and sensitization of the extrinsic pathway. In numerous studies involving cell line and cell line transplanted-induced tumors, BCP has shown chemopreventive and anticancer activity by thwarting the regression of tumors alone or in combination with conventional chemotherapeutics [13,182-186].

BCP isolated from several medicinal plants have shown significant chemopreventive and anticancer activity [70,187]. The efficacy and mechanism of plants showing anticancer activity attributed to BCP are represented in Table **5**. The anticancer mechanism of action of BCP is complex as it targets multiple factors in many cancer cell lines and cell line implanted tumor models of different types of cancer. These pathways include cell cycle progression, proliferation, apoptosis, angiogenesis, migration, invasion and metastasis of tumor in cancer models. Moreover, it inhibits oxidative damage of cellular components and inflammatory responses. BCP also modulates proteins, which is involved in metabolism processes [180-186]. A schematic representation of the mechanism of chemopreventive and anticancer activity of BCP is presented in Fig. (**6**).

Recently, Jung et al. [70] have demonstrated that BCP inhibits high-fat diet-induced melanoma progression in rats. The authors showed that feeding HFD stimulate solid tumor growth and lymph node (LN) metastasis in C57BL/6N mice injected with B16F10 melanoma cells. HFD feeding increased body weight gain, fasting blood glucose levels, solid tumor growth, LN metastasis, tumor cell proliferation, angiogenesis, and lymphangiogenesis, it decreased apoptotic cells, all of which were suppressed by dietary BCP. HFD feeding increased the number of lipid vacuoles and F4/80+ macrophage in tumor tissues and adipose tissues surrounding the LN. which was suppressed by BCP. HFD feeding increased the levels of CCL19 and CCL21 in the LN and the expression of CCR7 in the tumor; these changes were blocked by dietary BCP. In vitro culture results revealed that BCP inhibited lipid accumulation in 3T3-L1 preadipocytes; monocyte migration and MCP-1 secretion by B16F10s, adipocytes, and M2-MΦs; angiogenesis and lymph angiogenesis. The suppression of adipocyte and M2-cell accumulation and the inhibition of CCL19/21-CCR7 axis may be a part of mechanisms for the BCP suppression of HFD-stimulated melanoma progression. Costa et al. [187] reported that BCP isolated from Zornia brasiliensis, a popular folk medicine in Brazil exhibits cytotoxicity and inhibits proliferation against tumor cell lines from different histotypes using the Alamar blue assay.

Recently, BCP has isolated from *Aegle marmelos*, plant popularly known as Indian Bael showed anticancer properties in *in vitro* cell models; Jurkat and human neuroblastoma (IMR-32) cells. BCP caused down-regulation of anti-apoptotic genes (bcl-2, mdm2, cox2 and cmyb) and up-regulation of pro-apoptotic genes (bax, bak1, caspase-8, caspase-9 and ATM) in Jurkat and IMR-32 cells and revealed downstream apoptotic mechanism [13]. Further, docking (*in silico*) showed binding affinity of BCP to 15-lipoxygenase (15-LOX), which play an important role in the metabolism of arachidonic acid and represent an upstream target in attenuating inflammation [13]. The induction of apoptosis in lymphoma and neuroblastoma cells via modulation of 15-LOX implicates its role in the p53 mediate apoptosis of cell lines.

BCP isolated from clove has shown to cause induction of the detoxifying enzyme, GST in the mouse liver and small intestine [188]. This detoxifying property of BCP has been correlated with its anticarcinogenic potential [188]. Furthermore, the anticancer potential of BCP has been demonstrated against several solid tumor cell lines including breast cancer (MCF-7), prostate cancer (PC-3), lung cancer (A549), colorectal cancer (DLD-1), melanoma (M4BEU) and colon cancer (CT-26) [189]. Legault *et al.* [179] reported 10-fold augmenting effect of BCP on anticancer activity of paclitaxel against human tumor cell lines and found that even in low concentration inhibited cell growth. Several other studies during the recent past have provided ample evidences of anticancer and chemopreventive properties of BCP with different cell lines and models and the pharmacological actions described earlier

Plant containing BCP	Cytotoxic, antiproliferative/antitumor activity and mechanisms	References
Abies balsame	Breast (MCF-7), prostate (PC-3), lung (A549), colorectal (DLD-1), melanoma (M4BEU) and colon cancer (CT-26) cells	[179]
Eugenia caryophyllata	Anticarcinogenic activity by improving GST	[188]
Heterotheca inuloides	Several solid tumor cell lines	[189]
Platycladus orientalis	Amelanotic melanoma (C32) and renal cell adenocarcinoma cells	[190]
Prangosas perula Cupressus sempervirens		
Murraya koneigii	Modulates P-glycoprotein transport in Caco-2 (human colon carcinoma) cells	[183]
Didymocarpus tomentosa	HeLa tumor cells	[185]
Commiphora gileadensis	Antiproliferative activity in tumor cells and devoid of activity on normal cells	[180]
Salvia jamensis	Human leukemia cell lines (U937)	[191]
Aegle marmelos	Lymphoma (Jurkat) and human neuroblastoma (IMR-32) carcinoma cells	[13]
Neolitsea variabillima	Human oral, liver, lung, colon, melanoma, and leukemic cancer cells	[192]
Guatteriapo gonopus Martius	Human ovarian adenocarcinoma (OVCAR-8), human metastatic prostate cancer (PC-3M), human bronchoalveolar lung carcinoma (NCI-H358M) and antitumor activity in mice bearing sarcoma 180 tumor	[186]
Magnoliophyta	Human erythro leukemic cells (K562)	[193]
Heterotheca inuloides	Several solid tumor cell lines	[189]
Siegesbeckia orientalis	Lung cancer (A549), hepatoma (Hep G2), pharynx squamous (FaDu), breast (MDA-MB-231), prostate (LNCaP) and endometrial cancer (RL95-2) cells	[194]
Tagetes minuta Ocimum basilicum	Nasopharyngeal (KB) and hepatocellular carcinoma (HepG2) cells	[195]
Piper cernuum Vell.	B16F10-Nex2, U87, HeLa, HL-60, HCT and A2058 cells	[196]
Zornia brasiliensis	Tumor cell lines and B16-F10 mouse melanoma	[187]

Table 5. The cytotoxic and anticancer activities of β-Caryophyllene (BCP) in the *in vitro* and *in vivo* models of cancer.

[4,13,180-186]. Further mechanistic studies are needed to understand the molecular interaction and potential of BCP with dysregulated pathways associated with inflammation and cancer.

Chemotherapeutic agents which are used in the treatment of cancer exert dose-dependent severe multi organ toxicities and require the organ-protective adjuvants. Several of the drugs such as amifostine, dexerazoxon, leucovorin and erythropoietin are being used as organ protective adjuvants with chemotherapeutic agents. Numerous studies suggest the use of dietary antioxidants including natural bioactive agents as dietary adjuvants. In such efforts, BCP was investigated in in vivo animal models of cancer chemotherapy associated organ toxicities and showed to provide renoprotective effects against cisplatin-induced nephrotoxicity, doxorubicininduced cardiotoxicity and leukopenia, a hematologic disorder associated with chemotherapy. Following leukopenia in cancer patients the occurrence of secondary infection are common and affect the chemotherapy and causing numerous complications. BCP isolated from resinous oil extracted from the copaiba was demonstrated against secondary leukopenia, in an experimental chemotherapy model induced by 5-fluorouracil (5-FU) in Wistar rats [197].

Based on the findings of organ protective effects against chemotherapy associated organ toxicity, BCP appear an ideal, costeffective, safe adjuvant to cancer chemotherapy. It could protect normal body cells from cytotoxic actions and may sensitize the cancer cell to chemotherapeutic agent [15, 197]. Further, it may be a natural alternative to the synthetic agent to be better adjuvants to cancer therapy.

# 5.10. BCP in Atherosclerosis

Atherosclerosis is a vascular disorder involving inflammation, a narrowed vascular lumen in the entire tunica intima and reduced elasticity of the arterial wall. Cannabinoids are considered as key mediators in the pathophysiology of inflammatory diseases, including atherosclerosis [198]. Although only preliminary data have been reported on the activities of novel cannabinoid receptors, several studies have already investigated the role of CB1 and CB2 receptors in ischemic stroke [199]. While CB1 receptor activation has been shown to directly reduce atherosclerotic plaque inflammation, controversial data have shown neurotransmission and neuroprotection after stroke.

Given the potent anti-inflammatory activities on circulating leukocytes, the CB2 activation has been proven to produce protective effects against acute post-stroke inflammation [200]. In an *in vitro* study representing model of atherosclerosis, wherein HSP60 produced from Chlamydia pneumonia, mimics like human HSP60



Fig. (6). The anticancer mechanism of  $\beta$ -caryophyllene in different cancer cell lines and animal studies.

and induces vascular smooth muscle cells (VSMCs) growth and proliferation, a pathogenic process of atherosclerosis by attacking on immune and endothelial cells [201]. In this study, BCP has been shown to significantly inhibit HSP60-induced cell proliferation of VSMC even at low concentration [201]. The authors suggested that cyclic ring of BCP was considered responsible pharmacophore and favorable for interactions with receptors involved in the HSP60-induced VSMCs proliferation. However, further *in vitro* as well as *in vivo* studies are needed in order to translate therapeutic benefits and understand the CB2 mediated mechanism.

# 5.11. BCP in Respiratory Diseases

Natural remedies for the treatment of respiratory disorders are common practice in many parts of the world. A large number of people depend on the indigenous plant resources to treat various respiratory diseases like lungs disorders, asthma, bronchitis, common cold, cough and whooping cough [202]. A number of plants with medicinal importance are used to treat respiratory disorders in the area from generations to generations especially by rural population and forest ethnic communities [203]. Several plant extracts consisting BCP have shown beneficial in respiratory diseases including respiratory infections due to antiviral, antibacterial and spasmolytic activity [204,205].

The potential spasmolytic effect of BCP has shown on rat tracheal smooth muscle using the whole-cell voltage-clamp configuration of the patch-clamp technique measuring responses to K<sup>+</sup> depolarization and exposure to acetylcholine, respectively [206]. BCP showed potent blockade of electromechanical excitation-contraction coupling with minor inhibitory effect on pharmaco-mechanical coupling. Pre-exposure to BCP did not reduce ACh-induced contraction in isolated rat tracheal smooth muscle, regardless of the presence of intact epithelium. Inhibition of the inward Ba<sup>2+</sup> current by BCP showed that the antispasmodic activity on rat tracheal smooth muscle is mediated by the blockade of voltage-dependent  $Ca^{2+}$  channels and it could be a potential agent as spasmolytic in chronic inflammatory respiratory diseases. However, in the *in vivo* study in mice model of airways allergic inflammation, BCP did not exhibit preventive or therapeutic benefits as determined by leukocyte recruitment, pro-inflammatory cytokines and leukotriene levels in broncho-alveolar lavage fluid [207].

The medication popularly used to counteract cough is largely based on herbs or herb derived compounds. In context to another usage of BCP in respiratory diseases, a preliminary study has shown the antitussive and de-sputum effects of BCP in mice [208]. BCP alcohol is being used in preparing codeine as a medicament for relieving cough and/or dispersing phlegm with evident effects and apparent concentration-effect relationship.

# 5.12. BCP in Renal Diseases

Nephrotoxicity is one of the most important complications of cisplatin, a potent chemotherapeutic agent used for the treatment of various malignancies. There are intensive efforts to find out the effective less toxic alternatives or the adjuvant which could be prescribed with cisplatin to counter the nephrotoxicity involving oxidative stress and inflammation [209]. Cisplatin is known to cause dose dependent renal toxicity following chemotherapy and this animal model system is employed popularly to investigate the renoprotectives which may offer renal protection during chemotherapy. Additionally, the renal injury an invariable consequence of ischemia-reperfusion injury has been associated with alterations in renal functions and involves oxidative stress and inflammation. These alterations in renal functions are induced by both the ischemic insult and the release of oxygen-derived free radicals associated with the reperfusion process.

Recently, BCP has been showed to nephroprotection against renal toxicity induced by cisplatin, a pharmacological model of renal injury and results were further confirmed in genetically CB2 ablated mice model of renal toxicity to demonstrate CB2 receptor dependent mechanism [15]. BCP has been also showed to protect against renal toxicity in pharmacological model of renal toxicity and showed CB2 dependent mechanism in pharmacological as well as genetically CB2 ablated mice [15]. BCP dose-dependently ameliorated cisplatin-induced renal dysfunction, morphological damage, and inflammatory mediators (chemokines; MCP-1 and MIP-2, cytokines; TNF- $\alpha$  and IL-1 $\beta$ , adhesion molecule; ICAM-1, and neutrophil and macrophage infiltration) in kidneys. BCP treatment also mitigated oxidative/nitrative stress (NOX-2, -4 expression, 4-HNE and 3-NT content) and cell death. The absence of biochemical and histological markers of nephropathy in CB2 knockout mice further confirmed the protective effects of BCP and revealed that it may have potential as an adjunct to protect kidney against cisplatininduced renal injury. BCP treatment conferred renoprotection by mitigating oxidative/nitrosative stress, neutrophils and macrophage infiltration and apoptotic cell death.

In our laboratory, we evaluated the effect of BCP on glomerular and tubular renal functions following renal ischemia-reperfusion injury. BCP was tested for its effect on renal functional parameters and found to reduce proinflammatory cytokines, reduce lipid peroxidation and improve the antioxidant enzymes. Improved renal function along with preserved histological changes significantly demonstrates ameliorated ischemia-induced alterations in kidneys.

# 5.13. BCP in Liver Diseases

The liver diseases continue to be among the main threats to public health and they remain problems throughout the world. Despite enormous advances in modern medicine, there are no completely effective drugs that stimulate hepatic function, offer complete protection of the organ or help to regenerate hepatic cells. The advancement of medical science and technology is still unable to provide inclusive treatment to liver inflammation caused by either microbial invasion or antibiotics or environmental toxins [210]. Subsequently, because of the adverse effects of drug treatment, people start looking for comprehensive alternative nowadays. Herbal medicine is believed to be the best of choice because it is being practiced until now for centuries. Numerous herbal plants have been reported for their efficacies in liver protection and many phytochemicals derived from herbs were found promising as pharmaceutical alternatives for the treatment of liver diseases [211]. Several studies have evaluated the hepatoprotective activity of BCP in rat liver homogenates and in vivo [16, 68]. In the endogenous and induced tert-butyl hydroperoxide assays showed that BCP inhibits lipid peroxidation evidenced by reduced MDA formation mediated by free scavenger ability in relation to hydroxyl and superoxide anion radicals [82].

In another study, the protective effect of BCP on carbon tetrachloride-induced liver fibrosis and its inhibitory capacity on hepatic stellate cell (HSC) activation has been demonstrated [16]. BCP has been reported to reduce liver fibrosis and improve liver histology and reduce the expression of collagen-1, transforming growth factor beta 1 and tissue inhibitor matrix metalloproteinase-1 genes involved in tissue remodeling during tissue fibrosis and antioxidant effects. BCP also improved cell viability and reduced the expression of fibrotic marker genes in hepatic stellate cells activation model, wherein induction of oxidative stress leads overproduction of extracellular matrix proteins. BCP reducing MDA formation in both assays has been reported to have antioxidant activity, which explains its role as a potential hepatoprotective agent. The free radical scavenger activity in particular attenuation of hydroxyl and superoxide anion radicals and inhibition of the enzymes xanthine oxidase and 5-LOX, which are involved in the initiation of the lipid peroxidation, has been shown to contribute to the hepatoprotective activity of BCP [16].

Recently, the protective effects of BCP have been reconfirmed in a clinically relevant model of liver fibrosis induced by surgical ligation of the common bile duct [212]. In addition, it also ameliorated cholestasis-induced fibrosis and apoptosis mediated by cannabinoid receptors. Interestingly, BCP also diminished liver injury markers; serum ALT activity, serum AST, bilirubin levels, liver index CB1 and MMP-1 genes expression mediated via CB2 receptor activation. BCP activates CB2 receptor in HSCs and exerts antifibrotic effects [16]. Activation of CB2 receptor in Kupffer, endothelial and inflammatory cells attenuates inflammation and reactive oxygen and nitrogen species generation [213, 214]. It increased the number of Bcl2 positive hepatocytes and up-regulated MMP-1 gene expression. This may be responsible for the observed reduction of collagen deposition and hydroxyproline content in liver tissues.

Apart from, liver fibrosis, BCP isolated from Agastache rugosa, a medicinal plant popularly used in Korean folk medicine showed hepatoprotective against liver failure induced by d-galactosamine (GalN) and LPS in mice [215]. BCP injected intraperitoneally found to ameliorate rise in serum aminotransferase activity and IL-6 and TNF- $\alpha$  reduce mortality. Also, BCP has been found to reduce expression of TLR4 and receptor for advanced glycation end products (RAGE) protein expression, extracellular signal-related kinase, p38 and c-Jun N-terminal kinase phosphorylation, NF-kB, early growth response protein-1, and macrophage inflammatory protein-2 protein expression and level of high-mobility group box protein B1 (HMGB1). The HMGB1 is secreted from activated immune cells or passively released from injured cells. Excessive HMGB1 secretion/release adversely contributes to the pathogenesis of infectionand injury-elicited inflammatory diseases. The HMGB1 further activates immune cells to produce cytokines/chemokines through TLR4 or other receptors such as RAGE and TLR4 and can function either as a chemokine to stimulate leukocyte migration or as a cytokine to activate macrophages and endothelial cells to produce more cytokines, chemokines and adhesion molecules [216]. The mechanisms were further supported by similar observations in isolated Kupffer cells treated with LPS. BCP appears promising for hepatoprotection mediating down-regulation of the TLR4 and RAGE signaling.

In addition to the antioxidant [16] and anti-inflammatory activity [6], the high lipophilicity of BCP has been believed to facilitate the membrane stabilization and penetration across the cell membrane and explained its protective activity against lipid peroxidation. Based on the findings, the hepatoprotective effects of BCP were mediated solely via CB2 activation or by additional mechanisms like antioxidant or anti-inflammatory effects. The studies indicate that the selective pharmacological activation of the CB2 receptor is effective in attenuating hepatic fibrosis in liver disease.

# 5.14. BCP in Hyperpigmentation

Hyperpigmentation is commonly cared with therapeutic drugs or cosmetics of pigment-reducing or skin-whiten abilities. During melanogenesis, the process of generating pigmentation via melanin synthesis and delivery melanin synthesis processes, three key enzymes, tyrosinase, tyrosinase-related protein 1 (TRP1) and TRP2 metabolize melanin from L-tyrosine. Melanin synthesizing enzymes are regulated by microphthalmia-associated transcription factor (MITF). These two enzymes are responsible for melanin production and inhibition of them helps to attain a skin-whitening effect [217]. Many herbal medicines in particular flowers and their essential oils are effective in the treatment of hyperpigmentation [218]. However, many natural products used in preventive medicine have also been developed as cosmeceutical ingredients in skin care products with additional benefits such as antioxidant, cytotoxicity, skin irritation and anti-melanogenesis mechanisms [219].

Yang *et al.* [220] evaluated the main chemical components of the lime mint, which are used for cosmetics and in skin care products and the anti-melanogenic properties of its main component, BCP in B16F10 murine melanoma cells. The oil as well as BCP reduced melanin production in a dose-dependent manner by suppressing melanogenesis through the down-regulation of MITF, TRP-1, TRP-2 and tyrosinase. The benefits of BCP in skin care can be further compounded by a report of skin permeation and penetration of BCP and reaching in the stratum corneum, epidermis and dermis. BCP was used as skin penetration enhancers to promote the passage of compounds through the skin or cytoplasmic membrane [179, 221]. Given the benefits of multifunctional properties in

melanogenesis, inflammation and favorable application, BCP may be considered to be valuable future potential skin-whitening agents. Recently, the topically applied CB2 ligands have effectively attenuated contact allergic inflammation and benefit in atopic dermatitis. However, such data are lacking directly with BCP application and this could be investigated for the future development of strategies to harness BCP for the treatment of inflammatory skin diseases.

# 5.15. BCP in Infections

In recent decades, compounds derived from medicinal plants appear an important source of antimicrobial drugs and expanding considerably. The usage of antibiotics and antibacterial therapeutics is becoming less effective because of resistance to them and the appearance of adverse effects. Essential oils have been used since ancient times to treat infections [222]. Although there are numerous studies reporting the antimicrobial activity of essential oils, very few studies have focused on the activity of a single molecule. Additionally, the antimicrobial activities of these naturally occurring oils garnered attention in recent years, mainly in response to the overwhelming concern of consumers over the safety of synthetic food additives. The combinations of different types of essential oils or with other food additives have been found to potentially exhibit synergistic or additive effects. This provides a cost-efficient, economic and better alternative to both food industry and consumers, with simultaneous adhering to the hurdle technology in inhibiting proliferation of foodborne pathogens.

BCP is one of the phytochemicals largely used as a natural additive and preservatives in food products due to antimicrobial activity. BCP showed generalized antimicrobial effects against several microorganisms because of its capacity to interfere with cellular metabolism. BCP showed antimicrobial potential in several experimental studies including semi-solid agar susceptibility techniques. BCP showed inhibitory potential against bacterial, virus, fungi and protozoans [223-228]. In particular, BCP has been reported to possess effective bactericidal properties against oral pathogens and found effective as a natural anti-bacterial additive to protect foods from oxidative damages and foodborne pathogens [223-228]. The antibacterial activity against cariogenic bacteria, especially Streptococcus mutans were showed by BCP isolated from the essential oil extracted from the leaves of Plectranthus neochilus [43]. Recently, black pepper oil containing 30.33 % of BCP has shown potent antibacterial and antifungal activity and supported evidence on their use as food preservative by controlling microorganisms causing food-spoilage [40]. BCP present in Glechon spathulata and Glechon marifolia essential oil showed antifungal and antiherpes virus activity in Vero cells [226].

# 5.16. BCP in Parasitic Infestations

Numerous BCP-enriched plant extracts were also found effective against parasitic diseases such as malaria [229,230], ascariasis [86,231] and leishmaniasis [41, 55]. The larvicidal, mosquitocidal and mosquito repellent activity of BCP has also been reported [232]. BCP isolated from the aerial parts of Tanacetum argenteum found to exhibit potent larvicidal and bite deterrent activity against Aedes aegypti [233]. BCP isolated from the leaves of essential oil of Commiphora leptophloeos, a tree native to South America as well the oil found to exhibit potent concentration dependent oviposition deterrent effects and larvicidal action against A. aegypti, a vector of hemorrhagic fever dengue [234]. In another study, BCP isolated from the essential oil of Plectranthus barbatus, a plant of Ayurvedic importance has been found effective (50% lethal concentration (LC50) values) against larvae of the malaria vector Anopheles subpictus (41.66µg/ml), the dengue vector Aedes albopictus (44.77µg/ml) and the Japanese encephalitis vector Culex tritaeniorhynchus (48.17µg/ml) and appears a promising ecofriendly larvicidal agent [235]. BCP could be an alternative to synthetic insecticides, repellents and larvicideal agent and may be a potential safe and effective natural molecule for combating vector borne diseases [236-238]. The pinus species, *Pinus halepensis* and *Pinus pinaster* essential oil containing more than 22% BCP showed to potent larvicidal and repellent properties against *Aedes albopictus* [238]. Recently, BCP isolated from the essential oil of *Salvia leucantha* has shown larvicidal and biting-deterrent activity [236].

Further, BCP has also been found to exhibit *in vitro* antimalarial activity against the erythrocytic stages of chloroquine-sensitive strain of *Plasmodium falciparum* and cytotoxic effect against HeLa cells [229]. The histopathology findings revealed protection against nephrotoxicity of kidney, hepatic damage of liver and splenocytes protection. Additionally, BCP reduced parasitemia in mice by 88.2% against *Plasmodium berghei* [229]. The improved histopathology of kidney, liver and spleen in mice further demonstrated the protective property of BCP and suggested that BCP may be a safe and promising antimalarial compound.

BCP also showed a dose dependent leishmanicidal activity against intracellular amastigotes [55]. Recently, BCP isolated from the ethanolic extract of leaves of *Piper cernuum* has been found to decrease the parasitism by eradicating macrophages infected with *Leishmania amnazonensis* from infected and non-infected cells [196]. The leishmanicidal activity appeared to be independent of NO production by macrophages. In another study, BCP found effective against the strains of *Leishmania amazonensis* using mice peritoneal macrophage cells and comparable to pentamidine, a standard drug used for the treatment of leishmaniasis and ascariasis [41].

In another study, the nematicidal activity of BCP against Meloidogyne incognita has been demonstrated by docking to Glutathione S-transferases (GSTs) enzyme and assessing their binding affinity and inhibitory activity [86]. The GSTs are one of the major families of detoxifying enzymes, which detoxify different chemical compounds including insecticides in different insect species. The GST inhibitory activity could explain the nematocidal mechanism of BCP. A plethora of data from studies revealed that BCP may be an effective natural, safer and inexpensive candidate for future development in parasitic diseases such as malaria, leishmaniasis and ascariasis [41]. However, the mechanisms of the BCP antiparasiticidal activities are mainly based on in vitro or few in vivo studies using the whole extract containing a large proportion of BCP, further research using pure compound is required. Further studies required to demonstrate efficacy and main mechanisms by which BCP affect these organisms and may direct the development of a safe and natural compound for control of parasitic diseases.

#### 5.17. BCP as Insecticide in Pest Management

Chemical and biological treatments are generally used for insect control. However, in the past years, awareness on the toxicity of chemical treatments (use of pesticides), pest resurgence and resistance, and environmental pollution has prompted research towards finding green, safe and non-toxic applications in pest control. Some essential oils or their constituents have been suggested by many contributors as a source of alternative fumigants and insect repellents [237].

Plants produce a wide spectrum of volatile terpenoids that may mediate mutualistic or antagonistic interactions with other organisms numerous volatile, which have many different functions varying from plant hormones to abiotic stress protectants (e.g. heat protection) or in biotic stress responses (e.g. attraction of predators of plant herbivores) [238]. These compounds behave as semiochemicals that mediate the interactions of plants with other plants, animals and microorganisms and aid in to the plant survival under biotic and abiotic stresses. The volatiles function as cues for the attraction of pollinators [239] or as toxins for defense against herbivores [240]. Additionally, these volatiles can be used as signals that attract natural enemies of herbivores or for plant-plant communication [241].

A majority of these plant volatiles are terpenoids comprising over 40000 different structures [53]. Among them, the role of BCP in defense against pathogens has also been studied by manipulating the terpene synthase genes of Arabidopsis thaliana. It has been shown that BCP released from flowers, reduced infection by a bacterial pathogen [62,240]. BCP also acts as chemical messengers with diverse functions together with other organic compounds of essential oils [242]. BCP appears as a potential airborne signal, which participates in plant-plant communication. This occurs when plants receive volatiles from a damaged plant; the receivers become more resistant to herbivory [243]. Its presence in floral scents of plants appears as pollinator attraction [239]. It represents one of the major components (86%) of the volatile sex pheromones from Harmonia axyrides, an Asian lady beetle [244]. It also found to mediate below ground insect-plant interaction [245] and showed to impart resistance against herbivores [240]. BCP isolated from Artemisia argvi exhibited potent attracting activity relative to the standard, DEET. There are several plants known to consist of high content of BCP exhibiting antiparasitic [55], insect repellents and fumigants properties [3, 240]. BCP isolated from the essential oil of buds of Syzygium aromaticum popularly known as clove exhibited strong contact toxicity against the adults and nymphs of Cacopsylla chinensis, a pest of pears and appear a natural alternative to the conventional chemical pesticides [246]. BCP isolated from Hyptis suaveolens (Lamiaceae), a plant from Laos and Guinea-Bissau traditionally used as a mosquito repellent showed the tick repellent properties against nymphs of the tick, Ixodes ricinus. The oxidation or sulfidation of BCP exhibits enhanced tick repellent activity and revealed their potential application as tick repellent [247]. BCP isolated from the essential oil of Etlingera yunnanensis rhizomes and showed contact and repellent activities against Tribolium castaneum (Herbst) and Liposcelis bostrychophila (Badonnel) and revealed its potential as insecticide and repellent [248]. BCP extracted from the essential oil of Pogostemon cablin (Blanco) Bentham leaves showed insecticide and repellent activity against German cockroaches [249]. BCP isolated from the essential oil of aerial parts of Salvia ballotiflora (Lamiaceae) showed insectistatic and insecticidal activities against the fall armyworm Spodoptera frugiperda [250]. The studies revealed that the plants as well as BCP have potential to be developed into natural insecticides, fumigants or repellents in controlling insects and fungi in stored grains, natural products used for complementary medicine and traditional medicinal materials as an alternative to synthetic insecticides for pest management [251, 252]. These studies reveal the potential of BCP in integrated pest management program for the protection of crops as well as medicinal plants from worms and fungus by conferring resistance or manipulating the behavior and the plant essential oils containing high amount of BCP can potentially replace synthetic fungicides in the management of postharvest fruit and vegetable diseases [251].

#### 6. SAFETY AND TOXICITY OF BCP

Terpenes are an active class of natural compounds and considered safe and effective. BCP, a bicyclic sesquiterpene is commonly used in cosmetics, food & fragrances for the past several decades. The FDA has classified BCP as a flavoring substances and adjuvants which can be used in food at a minimum quantity either alone or with flavoring substances. It is designated "generally recognized as safe" by the USFDA and several other regulatory agencies worldwide for human consumption [6]. Recently, European Union (EU) has approved BCP for food preservation and adopted in EU for food additive legislation. It also showed to be as a biomarker for quality control and used widely for standardization and quality control of cosmeceuticals and pharmaceuticals containing medicinal plants rich in BCP.

It has been shown that the BCP is a common component of several thousand plants including vegetable, fruits and spices, thus the consumption of BCP as flavor, food preservative or additive is obviously common through human diet [6, 11, 253]. However, BCP is not generally consumed as a single entity rather it's ingested mostly as a component of diet through vegetables, spices, fruits and food plants [253]. This is indicative of its time tested safety in humans and supported by several reports mentioning it a dietary cannabinoid of common occurrence. In addition, a cosmetic preparation of fragrance oil containing BCP found safe on topical use [7].

The safety profile of BCP has been shown by several *in vitro* and *in vivo* studies revealing low toxicity for humans [83, 254-258]. Gertsch, 2008 [11] report it as a safe functional non-psychoactive and a macrocyclic anti-inflammatory CB2 receptor ligand in food-stuffs. The LD50 of acute oral doses of BCP in rats and the LD50 of acute dermal doses in rabbits found more than 5000 mg/kg [10]. BCP in intratracheal doses (12-48 mg/kg) was found non-toxic to the respiratory system including lungs in rats. Also, BCP in ointments upon dermal exposure did not found to cause skin irritation or sensitization in humans at concentrations up to 4% [256].

Most of findings in literature have reported absence of genotoxic or mutagenic effects with BCP in preclinical studies [83,255,257]. BCP did not cause mutagenic, genotoxic and bone marrow cytotoxic effects demonstrated by no effect on nuclear division index, frequency of micronuclei, proportions of polychromatic erythrocytes and reverse mutation assay in bacteria; Salmonella typhimurium and chemically induced genotoxicity [83,254-258]. Recently, the sesquiterpene compounds have attracted great interest because of their antimutagenic activity against common environmental pollutants [255,257]. The genotoxic potential and antimutagenic activity of BCP against discharged cigarette butts were investigated in the reverse mutation assay in bacteria [259]. The Salmonella typhimurium (TA98 and TA100 strains) and Escherichia coli (WP2uvrA strain) and S9 exogenous metabolic activator were used for the evaluation exogenous metabolic activation system S9 that mimics mammalian cytochrome P450 metabolism in the bacteria. BCP has been found to inhibit the mutagenicity of cigarette butts chemicals and reduced the revertant colonies induced by cigarette butts. When tested against the cigarette butt toxicants, BCP pre-, co- and post-treatment exhibited a strong antimutagenic effect. BCP also showed a strong antimutagenic activity against 2nitrofluorene and thwarted the mutagenicity of sodium azide, methyl methanesulfonate and 2-aminoanthracene. The antimutagenic activities of BCP further support its safety and efficacy long term use and its benefits in chemoprevention.

BCP acts as potent antimutagen compound by preventing DNA damage caused by the mutagens, while acted as desmutagens and bioantimutagens by interfering with fixation and progression of DNA damage in the intracellular compartment, enhancing the DNA repair and/or reversion systems. BCP elicits desmutagenic mechanisms by affecting the metabolic bioactivation of the procarcinogenic compounds such as heterocyclic aromatic amines contained in cigarette butt wherein the major chemicals are responsible for the mutagenicity of cigarette smoke. These chemical compounds during metabolism get activated by the enzymes of CYP450 family mainly CYP1A1, CYP1A2 and CYP1B1 into DNA reactive N-hydroxylamine which in turn cause genotoxicity [260]. BCP being lipophilic in nature interacts with the membrane of cells and amend the phospholipid structure, so interfere with the uptake of cigarette butt compounds into the cells. This was further supported by experiments employing permeation kinetics using multilamellar vesicles of dimyristoyl phosphatidylcholine wherein BCP binds to the phospholipid bilayer and diffuses across the biomembrane model [261]. The lipid peroxidation inhibiting activity further suggested the potential protective effect of BCP on the membrane phospholipids. Mechanistically, similar to vitamin E, BCP is believed to insert into membrane matrix due to its affinity to phospholipids, so stalling the initiation and progression of lipid peroxidation. Consequent to diminished lipid peroxidation, the membrane fragility is reduced and integrity is preserved by BCP treatment

[262]. The uptake of cigarette butt chemicals and toxicity reduced due to reduced permeability to toxicants. Additionally, BCP has also been found protective against environmental toxins in experimental models [15,16]. Several other studies have also shown that BCP is protective against environmental toxins in experimental models and that may contribute in inducing phase II detoxifying enzymes in particular Nrf2 enzymes.

Gertsch and colleagues [6,11] suggested that on average dietary intake of 10 to 200 mg of BCP may potentially modulate inflammatory and other pathophysiological processes where ECS plays a major role. BCP did not affect reproductive function such as conception, gestation, ovulation and different stages of pregnancy as well as delivery in rats [177]. BCP is broadly used in a number of cosmetic and pharmaceutical products which are applied topically to the skin intended for human use owing to its antipruritic, antiinflammatory, antiseptic and cooling properties. A vaginal cream of copaiba oleoresin containing high fractions of BCP found devoid of maternal toxicity, foetotoxicity and embryotoxicity at the doses applied which corresponds to ten times the recommended dose for use in humans [263]. This is suggestive of the safety of BCP on pregnancy and offspring's and indicates its safety during gestation. Upon oral ingestion, BCP has been detected in plasma and milk of the animals [264]. This reveals that BCP may reach into the mother's milk and therefore could expose infants nurtured on mother's milk in lactating mothers. Thus, the use of BCP in lactating woman may be cautioned.

In the pathological conditions, where BCP has been shown significant therapeutic and preventive potential, it has also been endowed with a relatively low toxicity in those animal studies and appears a very well-tolerated agent. In several experimental studies, the microscopic examination of the major organs and tissues apparently reveals absence of signs of toxicity, abnormal behavior, histopathological changes or any incidences of tumors compared to controls. Several preclinical studies have suggested that BCP selectively targets tumorigenic cells as opposed to non-tumorigenic cells and appears safe and tolerable in the experimental animals. In a preliminary study, BCP has been found to inhibit the ion channels particularly, Ca<sup>2+</sup> channels [206]. However, its physiological consequences and pharmacological benefits are still less understood and need to be investigated more in depth.

A majority of the pharmacological studies evaluated their potential either by oral or intraperitoneal route in the dose ranges from 5 to 500 mg/kg, body weight [67,79,93, 113,166]. The oral doses were reported readily bioavailable and showed response within an hour of administration [11]. The oral as well as intraperitoneal administration both were reported devoid of toxicity even at high doses from 5 mg/kg to 3000 mg/kg from days to months in different animal studies involving rats and mice [84,128,203]. Following exposure, BCP is reported to possess sensitization effect and may cause allergy [9,265]. It has been shown to oxidize quickly in air and forms caryophyllene oxide, which has been shown to cause photosensitivity or weak sensitizing capacity in the local lymph node assay [9].

Additionally, further studies should consider evaluating highly characterized extracts of medicinal plants standardized to contain BCP as one of the key components. This strategy has been employed for studying epigallocatechin-3-gallate (EGCG) in clinical trials through the use of a highly characterized green tea extract standardized to EGCG and other green tea polyphenols. This approach has also been recognized by the FDA as stated in their guideline entitled "guidance for industry-botanical drug products" and by the botanical drug products which receive investigational new drug status such as polyphenon E. The benefit of using a highly characterized and standardized extract would be cost effective and reasonable to procure BCP because of less purification. The other benefit will be that additional constituents found in extract which may confer synergistic action with BCP.

A majority of the plant extracts containing one third ingredient as BCP (represented in Table 1) were found well-tolerated and devoid of significant toxicity in animal studies. Though, there is lack of preclinical studies with BCP reporting the maximum tolerated dose, which is defined as the highest dose which is safe to administer in the absence of intolerable side effects. It could be regarded as a constraint in using BCP and determining the first dose size in humans for clinical trials. Further researches at both, the clinical and preclinical level are needed in order to determine the therapeutic effective dose of BCP in various diseases. In particular, the chronic safety and toxicity of BCP is still uncertain and should be investigated in future. Based on the time tested safety and demonstrated efficacy in different animal models of diseases, human studies with BCP are needed to translate the findings of animal studies in to humans.

# 7. PHARMACEUTICAL ANALYSES AND DEVELOPMENT OF BCP

BCP is relatively water-insoluble and sensitive to light, oxygen, humidity and high temperature and on air exposure auto-oxidize to caryophyllene oxide and it has been shown that 50% of the original compound get consumed after five weeks [9,266]. It gets epoxidized by molecular oxygen in the absence of any catalyst and also selectively isomerizes to isocaryophyllene under nitrogen atmosphere in the presence of a one-electron acceptor. The stereo selectivity of BCP and four conformations of BCP ( $\alpha\alpha$ ,  $\alpha\beta$ ,  $\beta\alpha$  and  $\beta\beta$ ) solvent effect (either chloroform or water) on the stability of the different conformers of BCP was studied and the  $\alpha\alpha$  conformer was predicted to be the most stable geometry [267]. The quantification methods for BCP in different plants mainly in Copaiba have been reported by several investigators [1,54,67,268-270].

BCP often exists in plants and plant based formulations in the enantiomeric composition and needs to be quantified and can be used a biomarker for the quality standards [52,55,270]. Patra *et al.* 2010 [270] developed a high performance thin layer chromatography method for the quantification of BCP in *amukkara choornam*, a polyherbal Siddha formulation. The solvent consists of toluene-ethyl acetate and BCP was detected at 260 nm. The developed method showed desired favorable linearity (R<sup>2</sup> = 0.9996 ± 0.0034), limit of detection (0.101 ng), limit of quantification (0.639 ng), accuracy (percentage recovery = 97.19 ± 1.204) and intraday and interday precisions (CV < 5%). The levels of BCP were reported 3.5-4.10 µg/gm in the herbal formulation.

The retention time for BCP standard is reported  $18.87\pm0.01$  min and for the BCP isolated from plant extract is  $18.94\pm0.03$  min. The tailing factor for BCP standard was  $1.05\pm0.01$  and BCP isolated from Copaiba was  $0.99\pm0.01$  with the peak purity for the BCP was  $1.00\pm0.00$ . Taken together, the methods demonstrate its specificity, linearity, precision and accuracy and considered an effective tool for assuring quality control of the essential oils containing BCP and appear a valid alternative to gas chromatography [56].

Although the percutaneous route of drug delivery has many advantages over other modes of delivery such as intravenous and oral administration, the architecture of the stratum corneum provides a formidable barrier to the topical and transdermal administration of some therapeutic agents [271]. To increase the permeability of the stratum corneum, several permeation enhancers are often used and continually tested for better agents. Being mostly non-irritant and non-toxic to the skin, they are commonly used to enhance the transdermal permeation of drugs such as 5-fluorouracil [272], propranolol [273], indomethacin and ketoprofen [274] as well as estradiol [275]. A recent study has shown that BCP could be developed as an effective penetration enhancer [54]. Recently, the essential oil of Rosemary containing BCP (3.8%) blended with topical diclofenac exhibited more pronounced analgesic effect due to enhanced transdermal percutaneous absorption of diclofenac in



Fig. (7). Yearly number of publication reports characterization, isolation or activity of  $\beta$ -caryophyllene in PUBMED.

the early as well as late phases as demonstrated by tail flick test and formalin tests [276].

In recent years, controlled drug delivery has been emerged as an active area of research and it has many potential technological applications. BCP could be an attractive candidate in drug delivery systems and nanoscience being used as rigid molecular framework for the drug delivery modes of controlled drug release [277]. Various strategies have been developed to achieve better transdermal delivery of certain compounds such as nicardipine and propanolol (for treatment of hypertension and angina) and ketoprofen and indomethacin (anti-inflammatory and analgesic drug) [278, 279]. Hence, BCP could be investigated to be utilized for transdermal drug delivery of above mentioned drugs.

Recently, Dias Dde et al. [56] have developed a nanoemulsion and showed that BCP penetrates through the skin due to the small droplet size and the high contact surface. The authors also developed a novel, sensitive, practical and solvent free method that uses gas chromatography in headspace mode coupled with mass spectrometry to evaluate the skin permeation/retention of BCP from the crude copaiba oil and its nanoemulsion. The method was fully validated, demonstrate linearity (r2 > 0.99), specificity (no peaks coeluting with BCP retention time), precision (RSD < 15%) and accuracy (recovery > 90%) within the accepted parameters and that the copaiba oil nanoemulsion presented a better skin penetration compared to the crude oil, with BCP achieving the most profound layer of the skin, the dermis. The mechanism by which BCP enhances drug permeation through the skin is not well-elucidated, but it could be presumed to be mediated by the disruption of the highly ordered lipid structure of the stratum corneum; the increase in drug diffusivity in the stratum corneum or increased drug partitioning into the stratum; and the increase in electrical conductivity of tissues thereby opening polar pathways within the stratum [280,281]. The evidences indicate that BCP could be one of the useful terpenes to achieve skin penetration enhancement.

# **CONCLUDING REMARKS & FUTURE PROSPECTS**

This review illustrate that BCP possess polypharmacological properties and its therapeutic potential has been revealed for several diseases including neuropathic pain, ulcerative colitis, liver diseases, cerebral ischemia, cancer, neurodegenerative and neuropsychiatric diseases including Alzheimer's, Parkinson's, Huntington's, Schizophrenia, epilepsy, mood disorders and alcohol addiction etc.

BCP being an important component of essential oils of many plants has been used since antiquity in traditional medicine to treat several ailments. Its application in diverse fields has been found ranging from the pharmaceutical and cosmetic industries to the food industries where it is used as an additive, flavor enhancer and preservative. It has received tremendous attention for commercial interest worldwide to be used in foods, cosmetics and fragrances intended for human use. Taking into account the low toxicity of this compound, it is not unexpected that it has a potential to garner interest and will sustain its popularity for pharmacological and pharmaceutical research. The available data indicate its inclusion either as prophylactic or adjuvant into the dietary management of chronic lifestyle diseases.

Molecular pharmacology data from several studies depict that BCP activates CB2 receptors, transcription factors; PPAR- $\gamma$  and PPAR- $\alpha$  isoforms, toll like receptor complex,  $\mu$ -opioid receptors and antagonizes  $\alpha$ 7-nicotinic acetylcholine receptors. In addition to modulate receptor function, BCP also modulates enzymatic, apoptotic and cell signaling pathways which play critical role in different therapeutic domains with significant therapeutic advantages. The multifunctional and polypharmacological properties also synergize the action of other drugs as demonstrated by activation of opioid receptors providing relief in neuropathic pain.

Though the available experimental evidences are promising, but there is still lack of information regarding translational aspects of BCP. The exact molecular mechanism by which this compound exerts its activity is also poorly understood. Furthermore, since BCP exists in various forms and pharmacophore appear modifiable, the structure-activity relationships need to be investigated in detail. The unique pharmacophore may also have potential to be developed as a blueprint for synthetically modified drugs. Medicinal products however are not yet available in market having only BCP. Huge amounts of BCP are produced annually in metric tons and it is clear that interest in this compound is not waning if one has to judge the number of publications related to BCP represented by Fig. (7). Apart from therapeutic potential, its potential as fumigants, lary-

#### Polypharmacological Properties and Therapeutic Potential of β-Caryophyllene

icidal, mosquitocidal and insect repellents also attracted the investigators hence it would be of interest to investigate extensively the potential effect of BCP against mosquito bite caused vector borne diseases such as malaria and dengue fever, the major killer diseases in many developing countries.

A significant number of CB2 receptor agonists such as JBT-101, KHK6188, ABT521, GRC10693, LY2828360, AZD1940, CR701, APD37D and S777469) progressed from Phase 1 to Phase 2 of clinical trials, mostly for the treatment of neuropathic pain, atopic dermatitis, postoperative neuralgia and osteoarthritis. Given the activity of BCP on interconnected multiple pharmacological and molecular signaling pathways, it can be reasonably speculated that BCP has the potential to be a polypharmacological multitargeted therapeutic agent. Moreover, investigating its interaction with other modern drugs could also advance the possibility of its synergistic combination with currently used drugs and may maximize the efficacy and minimize potential for the adverse effects.

Briefly, such an extended potent multi pharmacological actions and therapeutic application is rarely noted with a phytochemical. Knowing the pharmacological properties, favorable pharmacokinetics, high lipophilicity, wide therapeutic index along with high safety and relatively low toxicity profile provide substantial evidence for its future pharmaceutical development. Based on these favorable features, BCP appears a promising candidate for drug development. An impressive number of clinical trials are currently in progress to test the efficacy of CB2 receptors agonists of synthetic origin in various diseases. However, despite the potential tremendous benefit of this multifaceted dietary phytocannabinoid compound of natural origin, clinical trials are yet to be conducted for the translation of the animal findings in to humans.

	L	IST	OF	ABBRE	VIATIONS
--	---	-----	----	-------	----------

ACOX	=	acyl-CoA oxidase
ACS	_	acyl Co A synthetase
ACS	_	acyi-CoA synthetase
AST	=	Aspartate transaminase
AMPK	=	AMP-activated protein kinase
AUC	=	area under the curve
CREB	=	cAMP responsive element-binding protein
AD	=	Alzheimer's disease
AEDs	=	antiepileptic drugs
BDNF	=	brain-derived neurotrophic factor
BChE	=	butyrylcholinesterase
BCP	=	Beta-caryophyllene
Cmax	=	maximum concentration
COX-2	=	cyclooxygenase-2
CPT1	=	carnitine palmitoyl transferase
CD	=	Crohn's disease
CB1	=	cannabinoid receptor type 1
CB2	=	cannabinoid receptor type 2
DMARDs	=	Disease-modifying antirheumatic drugs
ECS	=	endocannabinoid system
EGCG	=	epigallocatechin-3-gallate
ERK	=	Extracellular Signal-Related Kinase
EFSA	=	European Food Safety Authority
FATP4	=	fatty acid transport protein 4
FAS	=	fatty acid synthase
FAAH	=	fatty acid amide hydrolase
IBD	=	Inflammatory bowel disease
TPSs	=	terpene synthases

Current Pharmaceutical Design, 2016, Vol. 22, No. 21 21
---

β-CD	=	β-cyclodextrin
GC-MS/SIM	=	gas chromatography coupled mass spec- trometry with selected ion monitoring mode
Tmax	=	time to peak plasma concentration
MRT	=	mean residence time
NSAIDS	=	Non-steroidal anti-inflammatory drugs
OA	=	osteoarthritis
PPARs	=	peroxisome proliferator-activated receptor
PPAR-γ	=	peroxisome proliferator-activated receptor gamma
α7-nAChRs	=	nicotinic acetylcholine receptors
TLR	=	toll-like receptor
T2DM	=	type 2 diabetes mellitus
TZDs	=	Thiazolidinediones
GSIC	=	glucose stimulated insulin secretion
SCD1	=	stearoyl-CoA desaturase
HSC	=	hepatic stellate cell
TRP1	=	tyrosinase-related protein 1
MITF	=	microphthalmia-associated transcription factor
PGE2	=	prostaglandin E2
iNOS	=	inducible nitric oxide synthase
NF-kB	=	NF-kappaB
MAP kinases	=	Mitogen-activated protein kinases
JNK	=	c-Jun N-terminal kinases
VSMCs	=	vascular smooth muscle cells
UC	=	ulcerative colitis

# **CONFLICT OF INTEREST**

The related works of the author are supported from grants provided by the College of Medicine and Health Sciences, UAE University, United Arab Emirates. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

# ACKNOWLEDGEMENTS

The authors acknowledge the financial support for the related research work by grants from the National Research Foundation (NRF) and United Arab Emirates University Program for Advanced Research (UPAR), United Arab Emirates. The authors also acknowledge a native English speaker Prof. Keith Bagnall, Professor of Anatomy, College of Medicine and Health Sciences, UAE University for the proof reading of the manuscript.

# REFERENCES

- [1] de Almeida Borges VR, Ribeiro AF, de Souza Anselmo C, Cabral LM, de Sousa VP. Development of a high performance liquid chromatography method for quantification of isomers betacaryophyllene and alpha-humulene in copaiba oleoresin using the Box-Behnken design. J Chromatogr B Analyt Technol Biomed Life Sci 2013; 940: 35-41.
- [2] Sales DS, Carmona F, de Azevedo BC, et al. Eugenia punicifolia (Kunth) DC. as an adjuvant treatment for type-2 diabetes mellitus: a non-controlled, pilot study. Phytother Res 2014; 28: 1816-21.
- [3] Khani A, Heydarian M. Fumigant and repellent properties of sesquiterpene-rich essential oil from Teucrium polium subsp. capitatum (L.). Asian Pac J Trop Med 2014; 7: 956-61.
- [4] Ricci D, Fraternale D, Giamperi L, et al. Chemical composition, antimicrobial and antioxidant activity of the essential oil of Teucrium marum (Lamiaceae). J Ethnopharmacol 2005; 98: 195-200.

- [5] Ghelardini C, Galeotti N, Di Cesare Mannelli L, Mazzanti G, Bartolini A. Local anaesthetic activity of beta-caryophyllene. Farmaco 2001; 56(5-7): 387-9.
- [6] Gertsch J, Leonti M, Raduner S, et al. Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci USA 2008; 105(26): 9099-104.
- [7] Bhatia SP, Letizia CS, Api AM. Fragrance material review on betacaryophyllene alcohol. Food Chem Toxicol 2008; 46(suppl 11): S95-6.
- [8] de Groot WN, Weyland JW, Nater JP. Unwanted Effects of Cosmetics and Drugs used in Dermatology. 3rd ed. USA: Elsevier 2015.
- [9] Skold M, Karlberg AT, Matura M, Borje A. The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. Food Chem Toxicol 2006; 44(4): 538-45.
- [10] Opdyke DL. Monographs on fragrance raw materials. Food Cosmet Toxicol 1973; 11: 1011-81.
- [11] Gertsch J. Anti-inflammatory cannabinoids in diet: Towards a better understanding of CB(2) receptor action? Commun Integr Biol 2008; 1: 26-8.
- [12] Donati M, Mondin A, Chen Z, et al. Radical scavenging and antimicrobial activities of Croton zehntneri, Pterodon emarginatus and Schinopsis brasiliensis essential oils and their major constituents: estragole, trans-anethole, beta-caryophyllene and myrcene. Nat Prod Res 2015; 29: 939-46.
- [13] Sain S, Naoghare PK, Devi SS, *et al.* Beta caryophyllene and caryophyllene oxide, isolated from Aegle marmelos, as the potent anti-inflammatory agents against lymphoma and neuroblastoma cells. Antiinflamm Antiallergy Agents Med Chem: United Arab Emirates 2014; 13(1): 45-55.
- [14] Hendriks H, Malingré TM, Batterman S, Bos R. Mono- and sesquiterpene hydrocarbons of the essential oil of Cannabis sativa. Phytochemistry 1975; 14: 814-5.
- [15] Horvath B, Mukhopadhyay P, Kechrid M, et al. beta-Caryophyllene ameliorates cisplatin-induced nephrotoxicity in a cannabinoid 2 receptor-dependent manner. Free Radic Biol Med 2012; 52(8): 1325-33.
- [16] Calleja MA, Vieites JM, Montero-Melendez T, Torres MI, Faus MJ, Gil A, Suarez A. The antioxidant effect of beta-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Br J Nutr 2013; 109(3): 394-401.
- [17] Alva M, Popich S, Borkosky S, Cartagena E, Bardon A. Bioactivity of the essential oil of an argentine collection of Acanthospermum hispidum (Asteraceae). Nat Prod Commun 2012; 7: 245-8.
- [18] Chai L, Lin CZ, Zhu CC, Zhao ZX, Zhang CX. [Analysis of the chemical constituents of essential oil from the leaves of Callicarpa integerrima]. Zhong Yao Cai 2010; 33: 382-5.
- [19] Thang TD, Dai do N, Luong NX, Ogunwande IA. Constituents of essential oils from the leaves, stem barks and resins of Canarium parvum Leen., and Canarium tramdenanum Dai et Yakovl. (Burseracea) grown in Vietnam. Nat Prod Res 2014; 28: 461-6.
- [20] Kobayashi C, Fontanive TO, Enzweiler BG, et al. Pharmacological evaluation of Copaifera multijuga oil in rats. Pharm Biol 2011; 49: 306-13.
- [21] Veiga Junior VF, Rosas EC, Carvalho MV, Henriques MG, Pinto AC. Chemical composition and anti-inflammatory activity of copaiba oils from Copaifera cearensis Huber ex Ducke, Copaifera reticulata Ducke and Copaifera multijuga Hayne--a comparative study. J Ethnopharmacol 2007; 112: 248-54.
- [22] Doria GA, Silva WJ, Carvalho GA, Alves PB, Cavalcanti SC. A study of the larvicidal activity of two Croton species from northeastern Brazil against Aedes aegypti. Pharm Biol 2010; 48: 615-20.
- [23] Rojas J, Baldovino S, Vizcaya M, Rojas LB, Morales A. The chemical composition of the essential oils of *Euphorbia caracasana* and *E. cotinifolia* (Euphorbiaceae) from Venezuela. Nat Prod Commun 2009; 4: 571-2.
- [24] Venturi CR, Danielli LJ, Klein F, et al. Chemical analysis and in vitro antiviral and antifungal activities of essential oils from Glechon spathulata and Glechon marifolia. Pharm Biol 2015; 53: 682-8.
- [25] Magalhaes LA, Lima Mda P, Marques MO, Facanali R, Pinto AC, Tadei WP. Chemical composition and larvicidal activity against Aedes aegypti larvae of essential oils from four Guarea species. Molecules 2010; 15: 5734-41.

- [26] Menezes Pdos P, Araujo AA, Doria GA, et al. Physicochemical characterization and analgesic effect of inclusion complexes of essential oil from Hyptis pectinata L. Poit leaves with betacyclodextrin. Curr Pharm Biotechnol 2015; 16: 440-50.
- [27] de Sousa EO, Rodrigues FF, Campos AR, Lima SG, da Costa JG. Chemical composition and synergistic interaction between aminoglycosides antibiotics and essential oil of Lantana montevidensis Briq. Nat Prod Res 2013; 27: 942-5.
- [28] Joshi RK. GC/MS analysis of the essential oil of Leucas indica from India. Nat Prod Commun 2014; 9: 1607-8.
- [29] Joshi RK. Leucas aspera (Willd.) Link Essential Oil from India: beta-Caryophyllene and 1-Octen-3-ol Chemotypes. J Chromatogr Sci. 2015 Nov 29. pii: bmv173.
- [30] Duarte Moreira RR, Zimmermann Martins G, Teixeira Botelho V, et al. Composition and activity against oral pathogens of the essential oil of Melampodium divaricatum (Rich.) DC. Chem Biodivers 2014; 11: 438-44.
- [31] Lebouvier N, Lawes D, Hnawia E, Page M, Brophy J, Nour M. The leaf, wood and bark oils of three species of Myodocarpus (Myodocarpaceae) endemic to New Caledonia. Nat Prod Commun 2014; 9: 1223-7.
- [32] Rodriguez EJ, Ramis-Ramos G, Heyden YV, et al. Chemical composition, antioxidant properties and antimicrobial activity of the essential oil of Murraya paniculata leaves from the mountains of Central Cuba. Nat Prod Commun 2012; 7: 1527-30.
- [33] Kang W, Li Y, Xu Y, Jiang W, Tao Y. Characterization of aroma compounds in Chinese bayberry (Myrica rubra Sieb. et Zucc.) by gas chromatography mass spectrometry (GC-MS) and olfactometry (GC-O). J Food Sci 2012; 77: C1030-5.
- [34] Olounlade PA, Azando EV, Hounzangbe-Adote MS, et al. In vitro anthelmintic activity of the essential oils of Zanthoxylum zanthoxyloides and Newbouldia laevis against Strongyloides ratti. Parasitol Res 2012; 110: 1427-33.
- [35] Ogunwande IA, Walker TM, Bansal A, Setzer WN, Essien EE. Essential oil constituents and biological activities of Peristrophe bicalyculata and Borreria verticillata. Nat Prod Commun 2010; 5: 1815-8.
- [36] Mohamadi M, Shamspur T, Mostafavi A. Comparison of the essential oil content and composition of the spathe, buds and pollen of Phoenix dactylifera. Nat Prod Res 2014; 28: 205-7.
- [37] Koutsaviti K, Giatropoulos A, Pitarokili D, Papachristos D, Michaelakis A, Tzakou O. Greek Pinus essential oils: larvicidal activity and repellency against Aedes albopictus (Diptera: Culicidae). Parasitol Res 2015; 114: 583-92.
- [38] Jirovetz L, Buchbauer G, Ngassoum MB, Geissler M. Aroma compound analysis of Piper nigrum and Piper guineense essential oils from Cameroon using solid-phase microextraction-gas chromatography, solid-phase microextraction-gas chromatographymass spectrometry and olfactometry. J Chromatogr A 2002; 976: 265-75.
- [39] Orav A, Stulova I, Kailas T, Müürisepp M. Effect of storage on the essential oil composition of Piper nigrum L. fruits of different ripening states. J Agric Food Chem 2004; 52(9): 2582-6.
- [40] Nikolić M, Stojković D, Glamočlija J, et al. Could essential oils of green and black pepper be used as food preservatives?. J Food Sci Technol 2015; 52(10): 6565-73.
- [41] Moura do Carmo DF, Amaral AC, Machado GM, Leon LL, Silva JR. Chemical and biological analyses of the essential oils and main constituents of Piper species. Molecules 2012; 17(2): 1819-29.
- [42] Padalia RC, Verma RS. Comparative study of volatile oil compositions of two Plectranthus species from northern India. Nat Prod Res 2011; 25: 1727-32.
- [43] Crevelin EJ, Caixeta SC, Dias HJ, et al. Antimicrobial activity of the essential oil of plectranthus neochilus against cariogenic bacteria. Evid Based Complement Alternat Med 2015; 2015: 102317.
- [44] Ozturk M, Duru ME, Aydogmus-Ozturk F, et al. GC-MS analysis and antimicrobial activity of essential oil of Stachys cretica subsp. smyrnaea. Nat Prod Commun 2009; 4: 109-14.
- [45] Boligon AA, Schwanz TG, Piana M, *et al.* Chemical composition and antioxidant activity of the essential oil of Tabernaemontana catharinensis A. DC. leaves. Nat Prod Res 2013; 27: 68-71.
- [46] Bezic N, Vuko E, Dunkic V, Ruscic M, Blazevic I, Burcul F. Antiphytoviral activity of sesquiterpene-rich essential oils from four croatian teucrium species. Molecules 2011; 16: 8119-29.

- [47] Hammami S, El Mokni R, Faidi K, et al. Chemical composition and antioxidant activity of essential oil from aerial parts of Teucrium flavum L. subsp. flavum growing spontaneously in Tunisia. Nat Prod Res 2015: 1-5.
- [48] Formisano C, Napolitano F, Rigano D, Arnold NA, Piozzi F, Senatore F. Essential oil composition of Teucrium divaricatum Sieb. ssp. villosum (Celak.) Rech. fil. growing wild in Lebanon. J Med Food 2010; 13: 1281-5.
- [49] Taherpour AA, Maroofi H, Bajelani O, Larijani K. Chemical composition of the essential oil of Valeriana alliariifolia Adams of Iran. Nat Prod Res 2010; 24: 973-8.
- [50] Joshi RK. Chemical composition of Vernonia albicans essential oil from India. Nat Prod Commun 2014; 9: 997-8.
- [51] Souza Barbosa PC, Moreira Wiedemann LS, da Silva Medeiros R, de Tarso Barbosa Sampaio P, Vieira G, Florencio da Veiga-Junior V. Phytochemical fingerprints of copaiba oils (Copaifera multijuga Hayne) determined by multivariate analysis. Chem Biodivers 2013; 10: 1350-60.
- [52] Tholl D, Kish CM, Orlova I, et al. Formation of monoterpenes in Antirrhinum majus and Clarkia breweri flowers involves heterodimeric geranyl diphosphate synthases. Plant Cell 2004; 16: 977-92.
- [53] Nikolić B, Ristić M, Bojović S, Krivošej Z, Matevski V, Marin PD. Population variability of essential oils of Pinus heldreichii from the Scardo-Pindic mountains Ošljak and Galičica. Chem Biodivers 2015; 12(2): 295-308.
- [54] Lucca LG, de Matos SP, Borille BT, et al. Determination of betacaryophyllene skin permeation/retention from crude copaiba oil (Copaifera multijuga Hayne) and respective oil-based nanoemulsion using a novel HS-GC/MS method. J Pharm Biomed Anal 2015; 104: 144-8.
- [55] Soares DC, Portella NA, Ramos MF, Siani AC, Saraiva EM. Transbeta -Caryophyllene: An Effective Antileishmanial Compound Found in Commercial Copaiba Oil (Copaifera spp.). Evid Based Complement Alternat Med 2013; 2013: 761323.
- [56] Dias Dde O, Colombo M, Kelmann RG, et al. Optimization of headspace solid-phase microextraction for analysis of betacaryophyllene in a nanoemulsion dosage form prepared with copaiba (Copaifera multijuga Hayne) oil. Anal Chim Acta 2012; 721: 79-84.
- [57] Budavari S, O'Neil M, Smith A, Heckelman P, Obenchain J. The Merck Index, Print Version. Twelfth ed. USA: CRC Press 1996.
- [58] Richter A, Seidl-Adams I, Kollner TG, Schaff C, Tumlinson JH, Degenhardt J. A small, differentially regulated family of farnesyl diphosphate synthases in maize (Zea mays) provides farnesyl diphosphate for the biosynthesis of herbivore-induced sesquiterpenes. Planta 2015; 241: 1351-61.
- [59] Yu ZX, Wang LJ, Zhao B, et al. Progressive Regulation of Sesquiterpene Biosynthesis in Arabidopsis and Patchouli (Pogostemon cablin) by the miR156-Targeted SPL Transcription Factors. Mol Plant 2015; 8(1): 98-110.
- [60] Robert CA, Erb M, Hiltpold I, et al. Genetically engineered maize plants reveal distinct costs and benefits of constitutive volatile emissions in the field. Plant Biotechnol J 2013; 11: 628-39.
- [61] Sultana N, Saify ZS. Enzymatic biotransformation of terpenes as bioactive agents. J Enzyme Inhib Med Chem 2013; 28: 1113-28.
- [62] Huang X, Xiao Y, Kollner TG, et al. Identification and characterization of (E)-beta-caryophyllene synthase and alpha/betapinene synthase potentially involved in constitutive and herbivoreinduced terpene formation in cotton. Plant Physiol Biochem 2013; 73: 302-8.
- [63] Oda S, Fujinuma K, Inoue A, Ohashi S. Synthesis of (-)-betacaryophyllene oxide via regio- and stereoselective endocyclic epoxidation of beta-caryophyllene with Nemania aenea SF 10099-1 in a liquid-liquid interface bioreactor (L-L IBR). J Biosci Bioeng 2011; 112(6): 561-5.
- [64] Matarese F, Cuzzola A, Scalabrelli G, D'Onofrio C. Expression of terpene synthase genes associated with the formation of volatiles in different organs of Vitis vinifera. Phytochemistry 2014; 105: 12-24.
- [65] Yahyaa M, Tholl D, Cormier G, Jensen R, Simon PW, Ibdah M. Identification and characterization of terpene synthases potentially involved in the formation of volatile terpenes in carrot (Daucus carota L.) roots. J Agric Food Chem 2015; 63(19): 4870-8.
- [66] Asakawa Y, Ishida T, Toyota M, Takemoto T. Terpenoid biotransformation in mammals. IV Biotransformation of (+)longifolene, (-)-caryophyllene, (-)-caryophyllene oxide, (-)-

#### Current Pharmaceutical Design, 2016, Vol. 22, No. 21 23

cyclocolorenone, (+)-nootkatone, (-)-elemol, (-)-abietic acid and (+)-dehydroabietic acid in rabbits. Xenobiotica 1986; 16: 753-67.

- [67] Liu H, Yang G, Tang Y, Cao D, Qi T, Qi Y, Fan G. Physicochemical characterization and pharmacokinetics evaluation of beta-caryophyllene/beta-cyclodextrin inclusion complex. Int J Pharm 2013; 450: 304-10.
- [68] Vinholes J, Rudnitskaya A, Goncalves P, Martel F, Coimbra MA, Rocha SM. Hepatoprotection of sesquiterpenoids: a quantitative structure-activity relationship (QSAR) approach. Food Chem 2014; 146: 78-84.
- [69] Chicca A, Caprioglio D, Minassi A, et al. Functionalization of betacaryophyllene generates novel polypharmacology in the endocannabinoid system. ACS Chem Biol 2014; 9: 1499-507.
- [70] Jung JI, Kim EJ, Kwon GT, et al. beta-Caryophyllene potently inhibits solid tumor growth and lymph node metastasis of B16F10 melanoma cells in high-fat diet-induced obese C57BL/6N mice. Carcinogenesis 2015; 36(9): 1028-39.
- [71] Bento AF, Marcon R, Dutra RC, Claudino RF, Cola M, Leite DF, Calixto JB. beta-Caryophyllene inhibits dextran sulfate sodiuminduced colitis in mice through CB2 receptor activation and PPARgamma pathway. Am J Pathol 2011; 178(3): 1153-66.
- [72] Felder CC, Joyce KE, Briley EM, et al. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 1995; 48(3): 443-50.
- [73] Wu C, Jia Y, Lee JH, Jun HJ, Lee HS, Hwang KY, Lee SJ. trans-Caryophyllene is a natural agonistic ligand for peroxisome proliferator-activated receptor-α. Bioorg Med Chem Lett 2014; 24(14): 3168-74.
- [74] Lee W, Ham J, Kwon HC, Kim YK, Kim SN. Anti-diabetic effect of amorphastilbol through PPARα/γ dual activation in db/db mice. Biochem Biophys Res Commun. 2013; 432(1): 73-9.
- [75] Guo K, Mou X, Huang J, Xiong N, Li H. Trans-caryophyllene suppresses hypoxia-induced neuroinflammatory responses by inhibiting NF-kappaB activation in microglia. J Mol Neurosci 2014; 54: 41-8.
- [76] Kota BP, Huang TH, Roufogalis BD. An overview on biological mechanisms of PPARs. Pharmacol Res 2005; 51(2): 85-94.
- [77] Inagaki T, Dutchak P, Zhao G, et al. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab 2007; 5(6): 415-25.
- [78] O'Sullivan SE1, Kendall DA. Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. Immunobiology 2010; 215(8): 611-6.
- [79] Paula-Freire LI, Andersen ML, Gama VS, Molska GR, Carlini EL. The oral administration of trans-caryophyllene attenuates acute and chronic pain in mice. Phytomedicine 2014; 21: 356-62.
- [80] Katsuyama S, Mizoguchi H, Kuwahata H, et al. Involvement of peripheral cannabinoid and opioid receptors in beta-caryophylleneinduced antinociception. Eur J Pain 2013; 17: 664-75.
- [81] Galdino PM, Nascimento MV, Florentino IF, et al. The anxiolyticlike effect of an essential oil derived from Spiranthera odoratissima A. St. Hil. leaves and its major component, beta-caryophyllene, in male mice. Prog Neuropsychopharmacol Biol Psychiatry 2012; 38: 276-84.
- [82] Vinholes J, Goncalves P, Martel F, Coimbra MA, Rocha SM. Assessment of the antioxidant and antiproliferative effects of sesquiterpenic compounds in *in vitro* Caco-2 cell models. Food Chem 2014; 156: 204-11.
- [83] Molina-Jasso D, Alvarez-Gonzalez I, Madrigal-Bujaidar E. Clastogenicity of beta-caryophyllene in mouse. Biol Pharm Bull 2009; 32(3): 520-2.
- [84] Alvarez-Gonzalez I, Madrigal-Bujaidar E, Castro-Garcia S. Antigenotoxic capacity of beta-caryophyllene in mouse, and evaluation of its antioxidant and GST induction activities. J Toxicol Sci 2014; 39: 849-59.
- [85] Hammami S, El Mokni R, Faidi K, et al. Chemical composition and antioxidant activity of essential oil from aerial parts of Teucrium flavum L. subsp. flavum growing spontaneously in Tunisia. Nat Prod Res 2015; 29(24): 2336-40.
- [86] Babu RO, Moorkoth D, Azeez S, Eapen SJ. Virtual screening and in vitro assay of potential drug like inhibitors from spices against Glutathione-S-Transferase of Meloidogyne incognita. Bioinformation 2012; 8(7): 319-25.
- [87] Assis LC, Straliotto MR, Engel D, Hort MA, Dutra RC, de Bem AF. beta-Caryophyllene protects the C6 glioma cells against

glutamate-induced excitotoxicity through the Nrf2 pathway. Neuroscience 2014; 279: 220-31.

- [88] Rufino AT, Ribeiro M, Sousa C, et al. Evaluation of the antiinflammatory, anti-catabolic and pro-anabolic effects of Ecaryophyllene, myrcene and limonene in a cell model of osteoarthritis. Eur J Pharmacol 2015; 750: 141-50.
- [89] Ma L, Jia J, Liu X, Bai F, Wang Q, Xiong L. Activation of murine microglial N9 cells is attenuated through cannabinoid receptor CB2 signaling. Biochem Biophys Res Commun 2015; 458: 92-7.
- [90] Fine PG, Rosenfeld MJ. The endocannabinoid system, cannabinoids, and pain. Rambam Maimonides Med J 2013; 4(4): e0022.
- [91] Fiorenzani P, Lamponi S, Magnani A, Ceccarelli I, Aloisi AM. In vitro and in vivo characterization of the new analgesic combination Beta-caryophyllene and docosahexaenoic Acid. Evid Based Complement Alternat Med 2014; 2014: 596312.
- [92] Zheng X, Sun T, Wang X. Activation of type 2 cannabinoid receptors (CB2R) promotes fatty acid oxidation through the SIRT1/PGC-1alpha pathway. Biochem Biophys Res Commun 2013; 436: 377-81.
- [93] Klauke AL, Racz I, Pradier B, et al. The cannabinoid CB(2) receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. Eur Neuropsychopharmacol 2014; 24: 608-20.
- [94] Al Mansouri S, Ojha S, Al Maamari E, Al Ameri M, Nurulain SM, Bahi A. The cannabinoid receptor 2 agonist, beta-caryophyllene, reduced voluntary alcohol intake and attenuated ethanol-induced place preference and sensitivity in mice. Pharmacol Biochem Behav 2014; 124: 260-8.
- [95] Suijun W, Zhen Y, Ying G, Yanfang W. A role for transcaryophyllene in the moderation of insulin secretion. Biochem Biophys Res Commun 2014; 444: 451-4.
- [96] Choi IY, Ju C, Anthony Jalin AM, Lee da I, Prather PL, Kim WK. Activation of cannabinoid CB2 receptor-mediated AMPK/CREB pathway reduces cerebral ischemic injury. Am J Pathol 2013; 182: 928-39.
- [97] Gertsch J. Immunomodulatory lipids in plants: plant fatty acid amides and the human endocannabinoid system. Planta Med 2008; 74: 638-50.
- [98] Okoye FB, Osadebe PO, Nworu CS, Okoye NN, Omeje EO, Esimone CO. Topical anti-inflammatory constituents of lipophilic leaf fractions of Alchornea floribunda and Alchornea cordifolia. Nat Prod Res 2011; 25: 1941-9.
- [99] Medeiros R, Passos GF, Vitor CE, et al. Effect of two active compounds obtained from the essential oil of Cordia verbenacea on the acute inflammatory responses elicited by LPS in the rat paw. Br J Pharmacol 2007; 151(5): 618-27.
- [100] Menezes Pdos P, Araujo AA, Doria GA, et al. Physicochemical characterization and analgesic effect of inclusion complexes of essential oil from Hyptis pectinata L. Poit leaves with betacyclodextrin. Curr Pharm Biotechnol 2015; 16: 440-50.
- [101] Ku CM, Lin JY. Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. Food Chem 2013; 141: 1104-13.
- [102] Galiegue S, Mary S, Marchand J, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 1995; 232: 54-61.
- [103] Choi JS, Park C, Jeong JW. AMP-activated protein kinase is activated in Parkinson's disease models mediated by 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine. Biochem Biophys Res Commun 2010; 391: 147-51.
- [104] Gowran A, Noonan J, Campbell VA. The multiplicity of action of cannabinoids: implications for treating neurodegeneration. CNS Neurosci Ther 2011; 17: 637-44.
- [105] Fernandez-Ruiz J, Moreno-Martet M, Rodriguez-Cueto C, et al. Prospects for cannabinoid therapies in basal ganglia disorders. Br J Pharmacol 2011; 163: 1365-78.
- [106] Zoppi S, Madrigal JL, Caso JR, et al. Regulatory role of the cannabinoid CB2 receptor in stress-induced neuroinflammation in mice. Br J Pharmacol 2014; 171: 2814-26.
- [107] Pacher P, Mackie K. Interplay of cannabinoid 2 (CB2) receptors with nitric oxide synthases, oxidative and nitrative stress, and cell death during remote neurodegeneration. J Mol Med (Berl) 2012; 90: 347-51.

- [108] Chang HJ, Kim JM, Lee JC, Kim WK, Chun HS. Protective effect of beta-caryophyllene, a natural bicyclic sesquiterpene, against cerebral ischemic injury. J Med Food 2013; 16: 471-80.
- [109] Chang HJ, Kim HJ, Chun HS. Quantitative structure-activity relationship (QSAR) for neuroprotective activity of terpenoids. Life Sci 2007; 80: 835-41.
- [110] Elmann A, Mordechay S, Rindner M, Larkov O, Elkabetz M, Ravid U. Protective Effects of the Essential Oil of Salvia fruticosa and Its Constituents on Astrocytic Susceptibility to Hydrogen Peroxide-Induced Cell Death. J Agric Food Chem 2009; 57: 6636-41.
- [111] Bonesi M, Menichini F, Tundis R, et al. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of Pinus species essential oils and their constituents. J Enzyme Inhib Med Chem 2010; 25: 622-8.
- [112] Murata K, Matsumura S, Yoshioka Y, Ueno Y, Matsuda H. Screening of beta-secretase and acetylcholinesterase inhibitors from plant resources. J Nat Med 2015; 69: 123-9.
- [113] Cheng Y, Dong Z, Liu S. beta-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 Mice through CB2 receptor activation and the PPARgamma pathway. Pharmacology 2014; 94: 1-12.
- [114] Anavi-Goffer S, Gertsch J. Treatment of schizophrenia using betacaryophyllene and cb2 receptor agonists, WO 2013140342 A1; PCT/IB2013/052182; Publication Date : 26 September 2013.
- [115] Aso E, Ferrer I. Cannabinoids for treatment of Alzheimer's disease: moving toward the clinic. Front Pharmacol 2014; 5: 37.
- [116] Jiang L, Role LW. Facilitation of cortico-amygdala synapses by nicotine: activity-dependent modulation of glutamatergic transmission. J Neurophysiol 2008; 99: 1988-99.
- [117] Parri HR, Hernandez CM, Dineley KT. Research update: Alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease. Biochem Pharmacol 2011; 82: 931-42.
- [118] King JR, Nordman JC, Bridges SP, Lin MK, Kabbani N. Identification and characterization of a G protein-binding cluster in alpha7 nicotinic acetylcholine receptors. J Biol Chem 2015.
- [119] Siddique YH, Naz F, Jyoti S, *et al.* Effect of centella asiatica leaf extract on the dietary supplementation in transgenic drosophila model of Parkinson's disease. Parkinsons Dis 2014; 2014: 262058.
- [120] dos Santos RG, Hallak JE, Leite JP, Zuardi AW, Crippa JA. Phytocannabinoids and epilepsy. J Clin Pharm Ther 2015; 40: 135-43.
- [121] Liu H, Song Z, Liao D, et al. Neuroprotective effects of transcaryophyllene against kainic acid induced seizure activity and oxidative stress in mice. Neurochem Res 2015; 40: 118-23.
- [122] Zarruk JG, Fernandez-Lopez D, Garcia-Yebenes I, et al. Cannabinoid type 2 receptor activation downregulates strokeinduced classic and alternative brain macrophage/microglial activation concomitant to neuroprotection. Stroke 2012; 43: 211-9.
- [123] Molina-Holgado E, Vela JM, Arevalo-Martin A, et al. Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. J Neurosci 2002; 22: 9742-53.
- [124] Ashton JC, Glass M. The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. Curr Neuropharmacol 2007; 5: 73-80.
- [125] Murikinati S, Juttler E, Keinert T, et al. Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. FASEB J 2010; 24: 788-98.
- [126] Suzuki N, Suzuki M, Hamajo K, Murakami K, Tsukamoto T, Shimojo M. Contribution of hypothermia and CB1 receptor activation to protective effects of TAK-937, a cannabinoid receptor agonist, in rat transient MCAO model. PLoS One 2012; 7: e40889.
- [127] Micale V, Di Marzo V, Sulcova A, Wotjak CT, Drago F. Endocannabinoid system and mood disorders: priming a target for new therapies. Pharmacol Ther 2013; 138: 18-37.
- [128] Bahi A, Al Mansouri S, Al Memari E, Al Ameri M, Nurulain SM, Ojha S. beta-Caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. Physiol Behav 2014; 135: 119-24.
- [129] Vasiljevik T, Franks LN, Ford BM, et al. Design, synthesis, and biological evaluation of aminoalkylindole derivatives as cannabinoid receptor ligands with potential for treatment of alcohol abuse. J Med Chem 2013; 56: 4537-50.
- [130] Basavarajappa BS. The endocannabinoid signaling system: a potential target for next-generation therapeutics for alcoholism. Mini Rev Med Chem 2007; 7: 769-79.

- [131] Baylac S, Racine P. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int J Aromatherapy 2003; 96: 138-42.
- [132] Merfort I. Perspectives on sesquiterpene lactones in inflammation and cancer. Curr Drug Targets 2011; 12: 1560-73.
- [133] Anand P, Whiteside G, Fowler CJ, Hohmann AG. Targeting CB2 receptors and the endocannabinoid system for the treatment of pain. Brain Res Rev 2009; 60: 255-66.
- [134] Ligresti A, Martos J, Wang J, *et al.* Prostamide F(2) alpha receptor antagonism combined with inhibition of FAAH may block the proinflammatory mediators formed following selective FAAH inhibition. Br J Pharmacol 2014; 171: 1408-19.
- [135] Whiteside TL. The role of death receptor ligands in shaping tumor microenvironment. Immunol Invest 2007; 36: 25-46.
- [136] Guindon J, Hohmann AG. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. Br J Pharmacol 2008; 153: 319-34.
- [137] Hsieh YH, McCartney K, Moore TA, et al. Intestinal ischemiareperfusion injury leads to inflammatory changes in the brain. Shock 2011; 36: 424-30.
- [138] Luongo L, Maione S, Di Marzo V. Endocannabinoids and neuropathic pain: focus on neuron-glia and endocannabinoidneurotrophin interactions. Eur J Neurosci 2014; 39: 401-8.
- [139] Wang D, Couture R, Hong Y. Activated microglia in the spinal cord underlies diabetic neuropathic pain. Eur J Pharmacol 2014; 728: 59-66.
- [140] Gui H, Tong Q, Qu W, Mao CM, Dai SM. The endocannabinoid system and its therapeutic implications in rheumatoid arthritis. Int Immunopharmacol 2015; 26: 86-91.
- [141] Ray K. Rheumatoid arthritis: Clue to role of cannabinoid receptors in RA. Nat Rev Rheumatol 2014; 10: 128.
- [142] Fukuda S, Kohsaka H, Takayasu A, et al. Cannabinoid receptor 2 as a potential therapeutic target in rheumatoid arthritis. BMC Musculoskelet Disord 2014; 15: 275.
- [143] Rom S, Persidsky Y. Cannabinoid receptor 2: potential role in immunomodulation and neuroinflammation. J Neuroimmune Pharmacol 2013; 8: 608-20.
- [144] Gertsch J, Raduner S, Altmann KH. New natural noncannabinoid ligands for cannabinoid type-2 (CB2) receptors. J Recept Signal Transduct Res 2006; 26(5-6): 709-30.
- [145] Agarwal RB, Rangari VD. Phytochemical investigation and evaluation of anti-inflammatory and anti-arthritic activities of essential oil of Strobilanthus ixiocephala Benth. Indian J Exp Biol 2003; 41: 890-4.
- [146] Fernandes ES, Passos GF, Medeiros R, et al. Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of Cordia verbenacea. Eur J Pharmacol 2007; 569: 228-36.
- [147] Camara CC, Nascimento NR, Macedo-Filho CL, Almeida FB, Fonteles MC. Antispasmodic effect of the essential oil of Plectranthus barbatus and some major constituents on the guineapig ileum. Planta Med 2003; 69: 1080-5.
- [148] Leonhardt V, Leal-Cardoso JH, Lahlou S, *et al.* Antispasmodic effects of essential oil of Pterodon polygalaeflorus and its main constituent beta-caryophyllene on rat isolated ileum. Fundam Clin Pharmacol 2010; 24(6): 749-58.
- [149] Ojo JO, Rezaie P, Gabbott PL, Stewart MG. Impact of age-related neuroglial cell responses on hippocampal deterioration. Front Aging Neurosci 2015; 7: 57.
- [150] Pant A, Saikia SK, Shukla V, Asthana J, Akhoon BA, Pandey R. Beta-caryophyllene modulates expression of stress response genes and mediates longevity in Caenorhabditis elegans. Exp Gerontol 2014; 57: 81-95.
- [151] Izzo AA, Coutts AA. Cannabinoids and the digestive tract. Handb Exp Pharmacol 2005: 573-98.
- [152] Di Sabatino A, Battista N, Biancheri P, et al. The endogenous cannabinoid system in the gut of patients with inflammatory bowel disease. Mucosal Immunol 2011; 4: 574-83.
- [153] Tourteau A, Andrzejak V, Body-Malapel M, et al. 3-Carboxamido-5-aryl-isoxazoles as new CB2 agonists for the treatment of colitis. Bioorg Med Chem 2013; 21: 5383-94.
- [154] Singh UP, Singh NP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS. Cannabinoid receptor-2 (CB2) agonist ameliorates colitis in IL-10(-/-) mice by attenuating the activation of T cells and promoting their apoptosis. Toxicol Appl Pharmacol 2012; 258: 256-67.

- [155] Slebioda TJ, Kmiec Z. Tumour necrosis factor superfamily members in the pathogenesis of inflammatory bowel disease. Mediatiors Inflamm 2014; 2014: 325129.
- [156] Cho JY, Chang HJ, Lee SK, Kim HJ, Hwang JK, Chun HS. Amelioration of dextran sulfate sodium-induced colitis in mice by oral administration of beta-caryophyllene, a sesquiterpene. Life Sci 2007; 80(10): 932-9.
- [157] Celinski K, Dworzanski T, Fornal R, et al. Comparison of antiinflammatory properties of peroxisome proliferator-activated receptor gamma agonists rosiglitazone and troglitazone in prophylactic treatment of experimental colitis. J Physiol Pharmacol 2013; 64: 587-95.
- [158] Annese V, Rogai F, Settesoldi A, Bagnoli S. PPARgamma in inflammatory bowel disease. PPAR Res 2012; 2012: 620839.
- [159] Nieto-Bobadilla MS, Siepmann F, Djouina M, et al. Controlled delivery of a new broad spectrum antibacterial agent against colitis: *In vitro* and *in vivo* performance. Eur J Pharm Biopharm 2015; 96: 152-61.
- [160] Lau JY, Barkun A, Fan DM, Kuipers EJ, Yang YS, Chan FK. Challenges in the management of acute peptic ulcer bleeding. Lancet 2013; 381: 2033-43.
- [161] Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S. Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, beta-caryophyllene. Planta Med 1996; 62: 469-70.
- [162] Wright MB, Bortolini M, Tadayyon M, Bopst M. Minireview: Challenges and opportunities in development of PPAR agonists. Mol Endocrinol 2014; 28: 1756-68.
- [163] Lefterova MI, Haakonsson AK, Lazar MA, Mandrup S. PPARgamma and the global map of adipogenesis and beyond. Trends Endocrinol Metab 2014; 25: 293-302.
- [164] Kim HL, Jeon YD, Park J, et al. Corni fructus containing formulation attenuates weight gain in mice with diet-induced obesity and regulates adipogenesis through AMPK. Evid Based Complement Alternat Med 2013; 2013: 423741.
- [165] Li C, Bowe JE, Jones PM, Persaud SJ. Expression and function of cannabinoid receptors in mouse islets. Islets 2010; 2: 293-302.
- [166] Basha RH, Sankaranarayanan C. beta-Caryophyllene, a natural sesquiterpene, modulates carbohydrate metabolism in streptozotocin-induced diabetic rats. Acta Histochem 2014; 116: 1469-79.
- [167] O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol 2007; 152: 576-82.
- [168] Stanley C, O'Sullivan SE. Vascular targets for cannabinoids: animal and human studies. Br J Pharmacol 2014; 171: 1361-78.
- [169] Aghamohammadi A, Hosseinimehr SJ. Antiangiogenic agents in natural products for the treatment of gynecologic disorders. Nutr Cancer 2014; 66: 206-13.
- [170] Munoz-Hernando L, Munoz-Gonzalez JL, Marqueta-Marques L, et al. Endometriosis: alternative methods of medical treatment. Int J Womens Health 2015; 7: 595-603.
- [171] Sanchez-Munoz BA, Aguilar MI, King-Diaz B, Rivero JF, Lotina-Hennsen B. The sesquiterpenes beta-caryophyllene and caryophyllene oxide isolated from Senecio salignus act as phytogrowth and photosynthesis inhibitors. Molecules 2012; 17(2): 1437-47.
- [172] Dmitrieva N, Nagabukuro H, Resuehr D, et al. Endocannabinoid involvement in endometriosis. Pain 2010; 151: 703-10.
- [173] Sasaki M, Hashimoto S, Sawa T, Amaya F. Tumor necrosis factoralpha induces expression of C/EBP-beta in primary afferent neurons following nerve injury. Neuroscience 2014; 279: 1-9.
- [174] Leconte M, Nicco C, Ngo C, et al. Antiproliferative effects of cannabinoid agonists on deep infiltrating endometriosis. Am J Pathol 2010; 177: 2963-70.
- [175] McHugh D, Page J, Dunn E, Bradshaw HB. Delta(9) -Tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. Br J Pharmacol 2012; 165: 2414-24.
- [176] McHugh D. GPR18 in microglia: implications for the CNS and endocannabinoid system signalling. Br J Pharmacol 2012; 167: 1575-82.
- [177] Abbas MA, Taha MO, Zihlif MA, Disi AM. beta-Caryophyllene causes regression of endometrial implants in a rat model of endometriosis without affecting fertility. Eur J Pharmacol 2013; 702: 12-9.

- [178] Ou MC, Hsu TF, Lai AC, Lin YT, Lin CC. Pain relief assessment by aromatic essential oil massage on outpatients with primary dysmenorrhea: a randomized, double-blind clinical trial. J Obstet Gynaecol Res 2012; 38: 817-22.
- [179] Legault J, Pichette A. Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. J Pharm Pharmacol 2007; 59: 1643-7.
- [180] Amiel E, Ofir R, Dudai N, Soloway E, Rabinsky T, Rachmilevitch S. beta-Caryophyllene, a compound isolated from the biblical balm of gilead (commiphora gileadensis), is a selective apoptosis inducer for tumor cell lines. Evid Based Complement Alternat Med 2012; 2012: 872394.
- [181] Chavan MJ, Wakte PS, Shinde DB. Analgesic and antiinflammatory activity of Caryophyllene oxide from Annona squamosa L. bark. Phytomedicine 2010; 17: 149-51.
- [182] da Silva KA, Paszcuk AF, Passos GF, et al. Activation of cannabinoid receptors by the pentacyclic triterpene alpha,betaamyrin inhibits inflammatory and neuropathic persistent pain in mice. Pain 2011; 152(8): 1872-87.
- [183] Zhang W, Lim LY. Effects of spice constituents on P-glycoproteinmediated transport and CYP3A4-mediated metabolism *in vitro*. Drug Metab Dispos 2008; 36(7): 1283-90.
- [184] Tundis R, Loizzo MR, Bonesi M, et al. In vitro cytotoxic effects of Senecio stabianus Lacaita (Asteraceae) on human cancer cell lines. Nat Prod Res 2009; 23(18): 1707-18.
- [185] Gowda PJ, Ramakrishnaiah H, Krishna V, Narra S, Jagannath N. Caryophyllene-rich essential oil of Didymocarpus tomentosa: chemical composition and cytotoxic activity. Nat Prod Commun 2012; 7: 1535-8.
- [186] do NFJE, Ferraz RP, Britto AC, et al. Antitumor effect of the essential oil from leaves of Guatteria pogonopus (Annonaceae). Chem Biodivers 2013; 10: 722-9.
- [187] Costa EV, Menezes LR, Rocha SL, et al. Antitumor Properties of the Leaf Essential Oil of Zornia brasiliensis. Planta Med 2015; 81: 563-7.
- [188] Zheng GQ, Kenney PM, Lam LK. Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. J Nat Prod 1992; 55: 999-1003.
- [189] Kubo I, Chaudhuri SK, Kubo Y, et al. Cytotoxic and antioxidative sesquiterpenoids from Heterotheca inuloides. Planta Med 1996; 62: 427-30.
- [190] Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA. Antiproliferative effects of essential oils and their major constituents in human renal adenocarcinoma and amelanotic melanoma cells. Cell Prolif 2008; 41(6): 1002-12.
- [191] Fraternale D, Albertini MC, Rudov A, et al. Compton: Trichomes, essential oil constituents and cytotoxic-apoptotic activity. Nat Prod Res 2013; 27: 1583-8.
- [192] Su YC, Hsu KP, Wang EI, Ho CL. Composition and *in vitro* anticancer activities of the leaf essential oil of Neolitsea variabillima from Taiwan. Nat Prod Commun 2013; 8: 531-2.
- [193] Lampronti I, Saab AM, Gambari R. Antiproliferative activity of essential oils derived from plants belonging to the Magnoliophyta division. Int J Oncol 2006; 29: 989-95.
- [194] Chang CC, Hsu HF, Huang KH, et al. Anti-proliferative effects of Siegesbeckia orientalis ethanol extract on human endometrial RL-95 cancer cells. Molecules 2014; 19(12): 19980-94.
- [195] Shirazi MT, Gholami H, Kavoosi G, Rowshan V, Tafsiry A. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of Tagetes minuta and Ocimum basilicum essential oils. Food Sci Nutr 2014; 2: 146-55.
- [196] Capello TM, Martins EG, de Farias CF, et al. Chemical composition and *in vitro* cytotoxic and antileishmanial activities of extract and essential oil from leaves of Piper cernuum. Nat Prod Commun 2015; 10(2): 285-8.
- [197] Campos MI, Vieira WD, Campos CN, Aarestrup FM, Aarestrup BJ. Atorvastatin and trans-caryophyllene for the prevention of leukopenia in an experimental chemotherapy model in Wistar rats. Mol Clin Oncol 2015; 3(4): 825-8.
- [198] Singla S, Sachdeva R, Mehta JL. Cannabinoids and atherosclerotic coronary heart disease. Clin Cardiol 2012; 35: 329-35.
- [199] Capettini LS, Savergnini SQ, da Silva RF, et al. Update on the role of cannabinoid receptors after ischemic stroke. Mediators Inflamm 2012; 2012: 824093.
- [200] Schmidt W, Schafer F, Striggow V, Frohlich K, Striggow F. Cannabinoid receptor subtypes 1 and 2 mediate long-lasting

neuroprotection and improve motor behavior deficits after transient focal cerebral ischemia. Neuroscience 2012; 227: 313-26.

- [201] Fukuoka K, Sawabe A, Sugimoto T, et al. Inhibitory actions of several natural products on proliferation of rat vascular smooth muscle cells induced by Hsp60 from Chlamydia pneumoniae J138. J Agric Food Chem 2004; 52: 6326-9.
- [202] Sorkness RL. CAM and respiratory disease. Nutr Clin Pract 2009; 24: 609-15.
- [203] Seebaluck R, Gurib-Fakim A, Mahomoodally F. Medicinal plants from the genus Acalypha (Euphorbiaceae)--a review of their ethnopharmacology and phytochemistry. J Ethnopharmacol 2015; 159: 137-57.
- [204] Wu QF, Wang W, Dai XY, et al. Chemical compositions and antiinfluenza activities of essential oils from Mosla dianthera. J Ethnopharmacol 2012; 139: 668-71.
- [205] Shah AJ, Rasheed M, Jabeen Q, et al. Chemical analysis and calcium channel blocking activity of the essential oil of Perovskia abrotanoides. Nat Prod Commun 2013; 8: 1633-6.
- [206] Pinho-da-Silva L, Mendes-Maia PV, Teofilo TM, et al. trans-Caryophyllene, a natural sesquiterpene, causes tracheal smooth muscle relaxation through blockade of voltage-dependent Ca(2)(+) channels. Molecules 2012; 17(10): 11965-77.
- [207] Rogerio AP, Andrade EL, Leite DF, Figueiredo CP, Calixto JB. Preventive and therapeutic anti-inflammatory properties of the sesquiterpene alpha-humulene in experimental airways allergic inflammation. Br J Pharmacol 2009; 158(4): 1074-87.
- [208] Tang XJ, Huang HR, Fang TZ, *et al.* The antitussive and de-sputum essential substance from the root of Gerbera piloselloides and its analytical compositions. Zhongguo Zhong Yao Za Zhi 2003; 28: 426-9.
- [209] Miller AA, Budzyn K, Sobey CG. Vascular dysfunction in cerebrovascular disease: mechanisms and therapeutic intervention. Clin Sci (Lond) 2010; 119: 1-17.
- [210] Zhang L, Schuppan D. Traditional Chinese Medicine (TCM) for fibrotic liver disease: hope and hype. J Hepatol 2014; 61: 166-8.
- [211] Zhang Z, Guo Y, Zhang S, *et al.* Curcumin modulates cannabinoid receptors in liver fibrosis *in vivo* and inhibits extracellular matrix expression in hepatic stellate cells by suppressing cannabinoid receptor type-1 *in vitro*. Eur J Pharmacol 2013; 721: 133-40.
- [212] Mahmoud MF, Swefy SE, Hasan RA, Ibrahim A. Role of cannabinoid receptors in hepatic fibrosis and apoptosis associated with bile duct ligation in rats. Eur J Pharmacol 2014; 742: 118-24.
- [213] Teixeira-Clerc F, Julien B, Grenard P, et al. CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. Nat Med 2006; 12: 671-6.
- [214] Pacher P, Gao B. Endocannabinoids and liver disease. III. Endocannabinoid effects on immune cells: implications for inflammatory liver diseases. Am J Physiol Gastrointest Liver Physiol 2008; 294: G850-4.
- [215] Cho HI, Hong JM, Choi JW, et al. beta-Caryophyllene alleviates dgalactosamine and lipopolysaccharide-induced hepatic injury through suppression of the TLR4 and RAGE signaling pathways. Eur J Pharmacol 2015; 764: 613-21.
- [216] Yu M, Wang H, Ding A, et al. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 2006; 26(2): 174-9.
- [217] Nicolaidou E, Katsambas AD. Pigmentation disorders: hyperpigmentation and hypopigmentation. Clin Dermatol 2014; 32: 66-72.
- [218] Fisk WA, Agbai O, Lev-Tov HA, Sivamani RK. The use of botanically derived agents for hyperpigmentation: a systematic review. J Am Acad Dermatol 2014; 70: 352-65.
- [219] Kim HJ, Kim IS, Dong Y, et al. Melanogenesis-inducing effect of cirsimaritin through increases in microphthalmia-associated transcription factor and tyrosinase expression. Int J Mol Sci 2015; 16: 8772-88.
- [220] Yang CH, Huang YC, Tsai ML, et al. Inhibition of melanogenesis by beta-caryophyllene from lime mint essential oil in mouse B16 melanoma cells. Int J Cosmet Sci 2015; 37: 550-4.
- [221] Cornwell PA, Barry BW. Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5fluorouracil. J Pharm Pharmacol 1994; 46: 261-9.
- [222] Seow YX, Yeo CR, Chung HL, Yuk HG. Plant essential oils as active antimicrobial agents. Crit Rev Food Sci Nutr 2014; 54: 625-44.
- [223] Bernardes WA, Lucarini R, Tozatti MG, et al. Antibacterial activity of the essential oil from Rosmarinus officinalis and its major

components against oral pathogens. Z Naturforsch C 2010; 65: 588-93.

- [224] Moon SE, Kim HY, Cha JD. Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. Arch Oral Biol 2011; 56(9): 907-16.
- [225] Kim YS, Park SJ, Lee EJ, et al. Antibacterial compounds from Rose Bengal-sensitized photooxidation of beta-caryophyllene. J Food Sci 2008; 73(7): C540-5.
- [226] Schmidt E, Bail S, Friedl SM, et al. Antimicrobial activities of single aroma compounds. Nat Prod Commun 2010; 5: 1365-8.
- [227] Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T. Screening chemical composition and *in vitro* antioxidant and antimicrobial activities of the essential oils from Origanum syriacum L. growing in Turkey. Biol Pharm Bull 2003; 26: 1725-9.
- [228] Lourens AC, Reddy D, Baser KH, Viljoen AM, Van Vuuren SF. In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. J Ethnopharmacol 2004; 95: 253-8.
- [229] Kamaraj C, Rahuman AA, Roopan SM, *et al.* Bioassay-guided isolation and characterization of active antiplasmodial compounds from Murraya koenigii extracts against Plasmodium falciparum and Plasmodium berghei. Parasitol Res 2014; 113: 1657-72.
- [230] Afoulous S, Ferhout H, Raoelison EG, *et al.* Chemical composition and anticancer, antiinflammatory, antioxidant and antimalarial activities of leaves essential oil of Cedrelopsis grevei. Food Chem Toxicol 2013; 56: 52-62.
- [231] Azeez S, Babu RO, Aykkal R, Narayanan R. Virtual screening and in vitro assay of potential drug like inhibitors from spices against glutathione-S-transferase of filarial nematodes. J Mol Model 2012; 18: 151-63.
- [232] Mansour SA, Messeha SS, el-Gengaihi SE. Botanical biocides. 4. Mosquitocidal activity of certain Thymus capitatus constituents. J Nat Toxins 2000; 9: 49-62.
- [233] Ali A, Tabanca N, Kurkcuoglu M, et al. Chemical composition, larvicidal, and biting deterrent activity of essential oils of two subspecies of Tanacetum argenteum (Asterales: Asteraceae) and individual constituents against Aedes aegypti (Diptera: Culicidae). J Med Entomol 2014; 51: 824-30.
- [234] da Silva RC, Milet-Pinheiro P, Bezerra da Silva PC, et al. (E)-Caryophyllene and alpha-Humulene: Aedes aegypti oviposition deterrents elucidated by gas chromatography-electrophysiological assay of Commiphora leptophloeos leaf oil. PLoS One 2015; 10(12): e0144586.
- [235] Govindarajan M, Rajeswary M, Hoti SL, Bhattacharyya A, Benelli G. Eugenol, alpha-pinene and beta-caryophyllene from Plectranthus barbatus essential oil as eco-friendly larvicides against malaria', dengue and Japanese encephalitis mosquito vector. Parasitol Res 2015 Oct 31.
- [236] Ali A, Tabanca N, Demirci B, et al. Chemical composition and biological activity of four salvia essential oils and individual compounds against two species of mosquitoes. J Agric Food Chem 2015; 63(2): 447-56.
- [237] Papachristos DP, Karamanoli KI, Stamopoulos DC, Menkissoglu-Spiroudi U. The relationship between the chemical composition of three essential oils and their insecticidal activity against Acanthoscelides obtectus (Say). Pest Manag Sci 2004; 60: 514-20.
- [238] Holopainen JK, Gershenzon J. Multiple stress factors and the emission of plant VOCs. Trends Plant Sci 2010; 15: 176-84.
- [239] Zito P, Dotterl S, Sajeva M. Floral volatiles in a sapromyiophilous plant and their importance in attracting house fly pollinators. J Chem Ecol 2015; 41: 340-9.
- [240] Fantaye CA, Kopke D, Gershenzon J, Degenhardt J. Restoring (E)beta-Caryophyllene Production in a Non-producing Maize Line Compromises its Resistance against the Fungus Colletotrichum graminicola. J Chem Ecol 2015; 41: 213-23.
- [241] Ruther J, Furstenau B. Emission of herbivore-induced volatiles in absence of a herbivore--response of Zea mays to green leaf volatiles and terpenoids. Z Naturforsch C 2005; 60: 743-56.
- [242] Kopke D, Beyaert I, Gershenzon J, Hilker M, Schmidt A. Speciesspecific responses of pine sesquiterpene synthases to sawfly oviposition. Phytochemistry 2010; 71: 909-17.
- [243] Shiojiri K, Ishizaki S, Ozawa R, Karban R. Airborne Signals of Communication in Sagebrush: A Pharmacological Approach. Plant Signal Behav 2015; 10(12): e1095416.

- [244] Fassotte B, Fischer C, Durieux D, Lognay G, Haubruge E, Francis F, Verheggen FJ. First evidence of a volatile sex pheromone in lady beetles. PLoS One 2014; 9: e115011.
- [245] Sato K, Takeuchi S. Chemical vapor detection using a reconstituted insect olfactory receptor complex. Angew Chem Int Ed Engl 2014; 53: 11798-802.
- [246] Tian BL, Liu QZ, Liu ZL, Li P, Wang JW. Insecticidal Potential of Clove Essential Oil and Its Constituents on Cacopsylla chinensis (Hemiptera: Psyllidae) in Laboratory and Field. J Econ Entomol 2015; 108(3): 957-61.
- [247] Ashitani T, Garboui SS, Schubert F, et al. Activity studies of sesquiterpene oxides and sulfides from the plant Hyptis suaveolens (Lamiaceae) and its repellency on Ixodes ricinus (Acari: Ixodidae). Exp Appl Acarol 2015; 67(4): 595-606.
- [248] Guo SS, You CX, Liang JY, et al. Chemical composition and bioactivities of the essential oil from etlingera yunnanensis against two stored product insects. Molecules 2015; 20(9): 15735-47.
- [249] Liu XC, Liu Q, Chen H, Liu QZ, Jiang SY, Liu ZL. Evaluation of contact toxicity and repellency of the essential oil of pogostemon cablin leaves and its constituents against blattella germanica (Blattodae: Blattelidae). J Med Entomol 2015; 52(1): 86-92.
- [250] Cárdenas-Ortega NC, González-Chávez MM, Figueroa-Brito R, et al. Composition of the Essential Oil of Salvia ballotiflora (Lamiaceae) and Its Insecticidal Activity. Molecules 2015; 20(5): 8048-59.
- [251] Elshafie HS, Mancini E, Sakr S, et al. Antifungal activity of some constituents of origanum vulgare l. essential oil against postharvest disease of peach fruit. J Med Food 2015; 18(8): 929-34.
- [252] Zhang WJ, You CX, Yang K, et al. Bioactivity of essential oil of Artemisia argyi Levl. et Van. and its main compounds against Lasioderma serricorne. J Oleo Sci 2014; 63: 829-37.
- [253] Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol 2011; 163: 1344-64.
- [254] Di Sotto A, Maffei F, Hrelia P, Castelli F, Sarpietro MG, Mazzanti G. Genotoxicity assessment of beta-caryophyllene oxide. Regul Toxicol Pharmacol 2013; 66: 264-8.
- [255] Di Sotto A, Mazzanti G, Carbone F, Hrelia P, Maffei F. Inhibition by beta-caryophyllene of ethyl methanesulfonate-induced clastogenicity in cultured human lymphocytes. Mutat Res 2010; 699(1-2): 23-8.
- [256] LaVoie EJ, Adams JD, Reinhardt J, Rivenson A, Hoffmann D. Toxicity studies on clove cigarette smoke and constituents of clove: determination of the LD50 of eugenol by intratracheal instillation in rats and hamsters. Arch Toxicol 1986; 59: 78-81.
- [257] Di Sotto A, Evandri MG, Mazzanti G. Antimutagenic and mutagenic activities of some terpenes in the bacterial reverse mutation assay. Mutat Res 2008; 653: 130-3.
- [258] Seifried HE, Seifried RM, Clarke JJ, Junghans TB, San RH. A compilation of two decades of mutagenicity test results with the Ames Salmonella typhimurium and L5178Y mouse lymphoma cell mutation assays. Chem Res Toxicol 2006; 19: 627-44.
- [259] Di Giacomo S, Mazzanti G, Di Sotto A. Mutagenicity of cigarette butt waste in the bacterial reverse mutation assay: The protective effects of beta-caryophyllene and beta-caryophyllene oxide. Environ Toxicol 2015.
- [260] Skipper PL, Kim MY, Sun HL, Wogan GN, Tannenbaum SR. Monocyclic aromatic amines as potential human carcinogens: old is new again. Carcinogenesis 2010; 31: 50-8.
- [261] Sarpietro MG, Di Sotto A, Accolla ML, Castelli F. Interaction of βcaryophyllene and β-caryophyllene oxide with phospholipid bilayers: Differential scanning calorimetry study. Thermochim Acta 2015; 600: 28-34.
- [262] Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: Mechanisms, inhibition, and biological effects. Biochem Biophys Res Commun 2005; 338: 668-76.
- [263] Lima CS, de Medeiros BJ, Favacho HA, et al. Pre-clinical validation of a vaginal cream containing copaiba oil (reproductive toxicology study). Phytomedicine 2011; 18: 1013-23.
- [264] Poulopoulou I, Zoidis E, Massouras T, Hadjigeorgiou I. Terpenes transfer to milk and cheese after oral administration to sheep fed indoors. J Anim Physiol Anim Nutr (Berl) 2012; 96: 172-81.
- [265] Paulsen E, Andersen KE. Colophonium and Compositae mix as markers of fragrance allergy: cross-reactivity between fragrance terpenes, colophonium and compositae plant extracts. Contact Dermatitis 2005; 53: 285-91.

- [266] Cevallos PAP, Buera MP, Elizalde BE. Encapsulation of cinnamon and thyme essential oils components (cinnamaldehyde and thymol) in Î<sup>2</sup>-cyclodextrin: Effect of interactions with water on complex stability. J Food Eng 2010; 99(1): 70-5.
- [267] Clericuzio M, Alagona G, Ghio C, Toma L. Ab initio and density functional evaluations of the molecular conformations of betacaryophyllene and 6-hydroxycaryophyllene. J Org Chem 2000; 65(21): 6910-6.
- [268] Sousa JP, Brancalion AP, Souza AB, et al. Validation of a gas chromatographic method to quantify sesquiterpenes in copaiba oils. J Pharm Biomed Anal 2011; 54: 653-9.
- [269] Gelmini F, Beretta G, Anselmi C, et al. GC-MS profiling of the phytochemical constituents of the oleoresin from Copaifera langsdorffii Desf. and a preliminary *in vivo* evaluation of its antipsoriatic effect. Int J Pharm 2013; 440: 170-8.
- [270] Patra KC, Singh B, Pareta S, Kumar KJ. A validated HPTLC method for determination of trans-caryophyllene from polyherbal formulations. Nat Prod Res 2010; 24: 1933-8.
- [271] Kalia YN, Naik A, Garrison J, Guy RH. Iontophoretic drug delivery. Adv Drug Deliv Rev 2004; 56: 619-58.
- [272] Williams AC, Barry BW. Essentail Oils as novel human skin penetration enhancers. Int J Pharm 1989; 721: R7-9.
- [273] Zhao K, Singh J. *In vitro* percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ethanol. J Control Release 1999; 62: 359-66.
- [274] Akitoshi Y, Takayama K, Machida Y, Nagai T. Effect of cyclohexanone derivatives on percutaneous absorption of ketoprofen and indomethacin. Drug Des Deliv 1988; 2: 239-45.

reserves the right to make minor modifications for further improvement of the manuscript.

Received: January 6, 2016

Accepted: March 10, 2016

DISCLAIMER: The above article has been published in Epub (ahead of print) on the basis of the materials provided by the author. The Editorial Department

- [275] Moghimi HR, Williams AC, Barry BW. A lamellar matrix model for stratum corneum intercellular lipids.1.Characterisation and comparison with stratum corneum intercellular structure. Int J Pharm 2015; 131(2): 103-15.
- [276] Akbari J, Saeedi M, Farzin D, Morteza-Semnani K, Esmaili Z. Transdermal absorption enhancing effect of the essential oil of Rosmarinus officinalis on percutaneous absorption of Na diclofenac from topical gel. Pharm Biol 2015; 53(10): 1442-7.
- [277] Bonan RF, Bonan PR, Batista AU, et al. In vitro antimicrobial activity of solution blow spun poly(lactic acid)/polyvinylpyrrolidone nanofibers loaded with Copaiba (Copaifera sp.) oil. Mater Sci Eng C Mater Biol Appl 2015; 48: 372-7.
- [278] O'Sullivan AJ, Crampton LJ, Freund J, Ho KK. The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. J Clin Invest 1998; 102: 1035-40.
- [279] Zahir A, Kunta JR, Khan MA, Reddy IK. Effect of menthol on permeability of an optically active and racemic propranolol across guinea pig skin. Drug Dev Ind Pharm 1998; 24: 875-8.
- [280] Vaddi HK, Ho PC, Chan SY. Terpenes in propylene glycol as skinpenetration enhancers: permeation and partition of haloperidol, Fourier transform infrared spectroscopy, and differential scanning calorimetry. J Pharm Sci 2002; 91: 1639-51.
- [281] Vaddi HK, Ho PC, Chan YW, Chan SY. Terpenes in ethanol: haloperidol permeation and partition through human skin and stratum corneum changes. J Control Release 2002; 81: 121-33.

Sharma et al.