

PipeNig[®], a black pepper (*Piper nigrum*) extract with standardized content of (*E*)- β -caryophyllene

A natural source of an endo-cannabinoid CB2 receptor agonist

➤ by Biosfered

The endogenous cannabinoid system plays an important role in the immune response to infection. At present, two cannabinoid (CB) receptors are described: cannabinoid type 1 receptor (CB1) and cannabinoid type 2 receptor (CB2), both G-protein coupled receptors. The CB2 receptor is the peripheral receptor for CBs, due to its expression on circulating immune cells. However, studies have also found CB2 expression in the brain, such as the cerebellum and microglial cells. The CB2 receptor is involved in the attenuation of inflammatory immune responses. CB2 receptor ligands have been shown to inhibit inflammation and oedema formation, exhibit analgesic effects, play a protective role in hepatic ischaemia–reperfusion injury, and prevent experimental colitis by reducing inflammation.

CB2 receptor pathway activation suppresses cytokine release from immune cells, thereby dampening the inflammatory response (immunosuppression) [1]. CB2 receptor-selective agonists which lack the psychoactive side effects typically associated with CB1 receptor activation are potential candidates as a valid support in different diseases. (*E*)- β -Caryophyllene (BCP) (Fig. 1) is a sesquiterpene hydrocarbon that selectively binds to the CB2 receptor and is a functional CB2 agonist [2]. BCP exerts toxicity at doses above 2,000 mg/kg body weight and also has anxiolytic-like and anti-depressant effects. The possibility that BCP may ameliorate mood disorders offers exciting prospects for future studies [3].

BCP was also found to be an excellent therapeutic agent to prevent cisplatin-induced nephrotoxicity through a CB2 receptor-dependent pathway and has potential efficacy in preventing and ameliorating non-alcoholic fatty liver disease and its associated metabolic disorders. Finally, BCP possesses antioxidant properties, preventing lipidic oxidative damage and enhancing the activity of glutathione peroxidase, an important enzyme linked to the prevention of atherosclerosis. PipeNig[®] is a black pepper (*Piper nigrum*) (Fig. 2) liquid or powder extract produced by Biosfered (Turin, Italy) that possesses the highest content of bioactive BCP available on the market.

Composition and technical specifications

PipeNig[®] is produced by Biosfered using a proprietary and patented production method. It is characterized by a high standardized content of bioactive (*E*)- β -Caryophyllene (identified by GC-MS and quantified by GC-FID). PipeNig[®] is available as a powder extract (PipeNig[®]-PWD) and a liquid extract (PipeNig[®]-FL). Both products are standardized and titrated to provide 800 g bioactive (*E*)- β -Caryophyllene (PipeNig[®]-FL) and 300 g bioactive (*E*)- β -Caryophyllene (PipeNig[®]-PWD) per kg of product. PipeNig[®] is stable at room temperature. The technical specifications of PipeNig[®]-PWD and PipeNig[®]-FL are reported in Table 1.

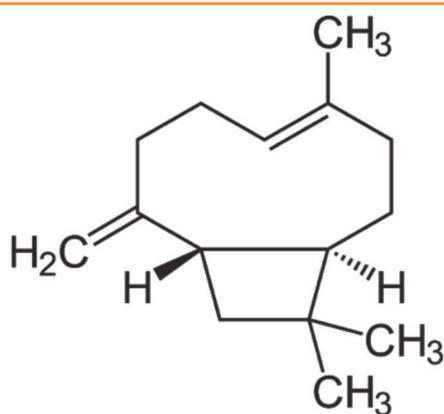


Figure 1 - Chemical formula of (*E*)- β -caryophyllene present in PipeNig[®]

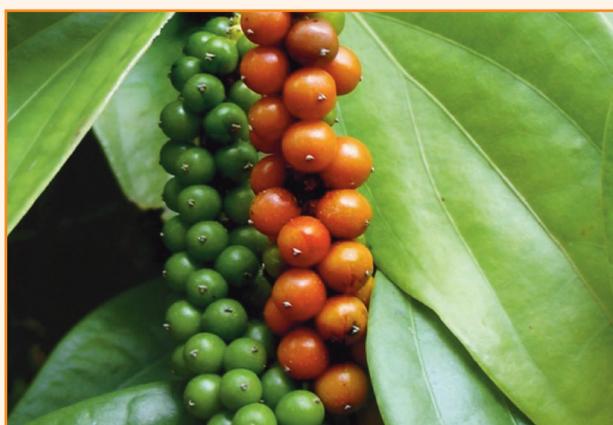


Figure 2 - *Piper nigrum* berries on a wild plant in Vietnam

	PipeNig®-PWD	PipeNig®-FL
Organoleptic properties		
Appearance	Powder (≥60 mesh)	Liquid
Colour	White powder	Colourless liquid
Physical and chemical properties		
Extraction solvent	Water	Water
Total (E)-β-caryophyllene content (g/kg)	≥300	≥800
Other compounds (g/kg)	≤25	≤65
Loss on drying (%)	≤9	<1
Tapped bulk density (kg/m ³)	525–545	Not applicable
Contaminants		
Residual organic solvents		
Ethanol (%)	≤1	Absent
Methanol (ppm)	<10	Absent
Heavy metals (ppm)		
Pb	<3	<3
Cd	<1	<1
Hg	<0.1	<0.1
As	<1	<1
Aflatoxins (B1, B2, G1, G2) (ppb)	<10	<10
Total aflatoxin B1 (ppb)	<5	<5
Ochratoxin A (Reg. 1881/2006 and amendments) (ppb)	<15	<15
Melamin (Reg. 1881/2006 and amendments) (ppm)	<2.5	<2.5
Pesticide residues	In accordance with EC No. 396/2005 and subs. amend	
Polycyclic aromatic hydrocarbons (PAH) (Reg. 1933/2015)		
Benzo[a]pirene (ppb)	<10	<10
Sum of all other PAH (ppb)	<50	<50
Excipients		
Rice (<i>Oryza sativa</i> L.) (%)	Rice starch <70	Rice oil <20
Auxiliary substances	Absent	Absent
Preservatives	Absent	Absent
Gluten (ppm)	Absent	Absent
Microbiological properties		
Total aerobic microbial count (CFU/g)	<5×10 ⁴	<5×10 ⁴
Total yeast microbial count (CFU/g)	<100	<100
Enterobacteriaceae	Absent	Absent
<i>Salmonella</i> spp	Absent	Absent
<i>Escherichia coli</i>	Absent	Absent
<i>Staphylococcus aureus</i>	Absent	Absent
Storage	In a cool, dry, ventilated area away from direct light	
Shelf-life	24 Months	
Allergens	Absent	
Other specifications	GMO-free, no nanomaterials, non-irradiated, vegan-compliant, WADA compliant	

Table 1 - Technical specifications of PipeNig®-PWD and PipeNig®-FL

Efficacy

Mechanism of action and preclinical studies

BCP is the first cannabis-derived functional CB receptor ligand with a fundamentally different structure from the classic cannabinoids. BCP selectively binds to the binding site CP55.940 of CB2 (THC binding site), leading to cell activation and anti-inflammatory effects. BCP binds to the hydrophobic region of the receptor. This binding leads to inhibition of adenylate cyclase and therefore inhibition of the conversion of ATP to cyclic AMP (cAMP). BCP weakly induces p38 and Erk1/2 phosphorylation in primary CD14+ monocytes and, at the same time, inhibits LPS-stimulated TNF-α and IL-1β protein expression in whole blood.

Since the signalling pathways Erk1/2 and JNK1/2 are fundamental for the expression of IL-1 and TNF-α, the CB2 receptor ligand BCP inhibits the activation of these kinases and down-regulates the expression of IL-1 and TNF-α. Moreover, the MAPK pathway allows information transmitted by BCP to arrive directly into the nucleus, significantly inhibiting the production of IL-1 and TNF-α [2, 4]. In vitro and in vivo pre-clinical studies on BCP demonstrate that its biological effects include anti-inflammatory, antimicrobial, antioxidant and analgesic activity. These biological effects derive from the ability of BCP to activate the CB2 endo-cannabinoid receptor, thus behaving as an endo-cannabinoid. The specificity of BCP for the CB2 receptor, mainly expressed in peripheral tissues, and its inability to bind CB1, which is predominantly expressed at the level of the central nervous system, implies that its action is devoid of the known psychoactive effects associated with the activation of CB1, thus suggesting its potential use as a painkiller and/or anti-inflammatory as an interesting alternative to cannabis. The analgesic efficacy of BCP for chronic and acute pain has been evaluated in vivo against placebo and against morphine [5]. The results showed a dose-dependent analgesic action in mice with dosages of 5 mg/kg and 10 mg/kg for acute and chronic pain, respectively. Comparison with morphine and with cannabinoid and opioid antagonists (naloxone) showed that the analgesic effect of BCP resulted from action on both the cannabinoid and the opioid receptors. Recent findings indicate there are significant implications for clinical research and strongly support the effectiveness of BCP as a novel molecule to investigate as a therapeutic agent for multiple sclerosis [6].

Human pilot study

In a pilot study performed by the Farmacia Centrale (Cam-

biano, Italy), a balanced sample of 31 volunteers (19 females and 12 males, aged 36 to >76 years) with either acute (48%) or chronic (52%) pain, including headache (8%), cervical pain (20%), joint pain (32%), muscle aches (16%), lower back pain (12%) and other pain (12%), was studied for 2 months and PipeNig®

compared with different NSAIDs. The volunteers received two capsules/day containing 100 mg of PipeNig® (corresponding to 30 mg of bioactive BCP) for 10 days or until pain relief. A score was recorded for all volunteers based on a questionnaire reporting their direct experience with PipeNig®. In general, volunteers reported a 60% reduction in pain between the third and fourth day after initiation of treatment (Fig. 3A) and the perceived effect was similar or slightly lower than that of the NSAID used for 38% and 31% of the volunteers, respectively (Fig. 3B). Finally, when volunteers were asked whether they would regularly use PipeNig®, more than 70% said they would (Fig. 3C).

Safety

PipeNig® is produced under strict procedures and the safety of the product is guaranteed by advanced technology detection systems (microbiological, chemical and molecular).

Application and use

Careful authentication of the bioactive BCP by GC-MS and quantification and standardization by GC-FID are necessary for preparing effective analgesic doses. Our pilot study indicates that PipeNig®-PWD and PipeNig®-FL are attractive candidates for the development of novel natural painkillers. The recommended dosage of PipeNig®-PWD is 100 mg/dose. This dosage has been demonstrated to be effective when administered once a day for at least 10 days.

PipeNig®-FL is an alcohol-free liquid source of BCP with the highest content (800 g/kg standardized BCP) available on the market and is particularly suitable for all liquid applications, including soft gels.

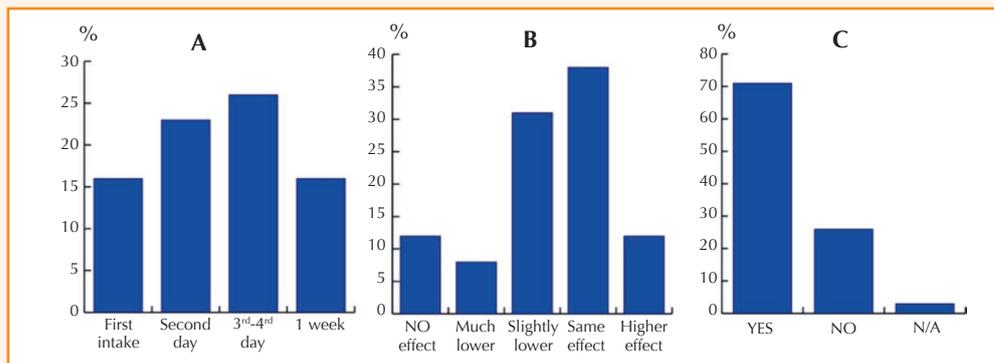


Figure 3 - Result of the pilot study performed by the Farmacia Centrale (Cambiano, Italy) on a balanced sample of 31 volunteers with acute or chronic pain after administration of PipeNig® as compared to different NSAIDs. (A) Timing of pain reduction after administration of PipeNig®. (B) Perception of the effect of PipeNig® as compared with the NSAID usually taken to reduce pain. (C) Response of volunteers to the question whether they would use again PipeNig® as a painkiller. Values are expressed as percentage of responses.

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Biosfered in a nutshell

Biosfered S.r.l. is an Italian company that produces liquid and powder extracts from plant matrices obtained through patented techniques and technologies based on green chemistry that do not involve the use of toxic solvents. The products are chemically characterized and titrated using the most advanced analytical and mass spectrometric techniques (GC-MS, HPLC-ESI-MS/MS, MALDI-qTOF). The reference market consists of the pharmaceutical, nutraceutical, food and cosmetic industries.

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