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CAFFEIC ACID PHENETHYL ESTER (CAPE) – NATURAL SOURCES, ANALYTICAL PROCEDURES AND SYNTHETIC APPROACHES

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Mini Review
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Abstract

Caffeic acid phenethyl ester is the most studied of all the individual constituents of propolis. It possesses diverse and potent pharmacological activities: anticancer, antibacterial, antiviral, anti-inflammatory, immunomodulating, cytotoxic, neuroprotective, hepatoprotective, cardioprotective, etc. The interest in the versatile pharmacological activities of CAPE led to a significant number of publications dealing with different ways of synthesis of caffeic acid phenethyl ester, last reviewed in 2014. Natural sources of CAPE and methods of analysis of this important secondary metabolite have not been reviewed yet. That is why the present review summarizes the available literature data on CAPE natural sources, analytical techniques for determination of CAPE in different matrices, and latest development in synthetic approaches (newly published after 2014).

Key words: caffeic acid phenethyl ester, CAPE, synthesis, plant sources, analysis

Introduction. Caffeic acid phenethyl ester (CAPE) is a relatively simple natural compound (Fig. 1). It is somewhat surprising that it has been described for the first time in the scientific literature as late as 1988. At that time it has been isolated from bee propolis as the carcinostatic active principle and found to possess preferential cytotoxicity on tumour cells [1].

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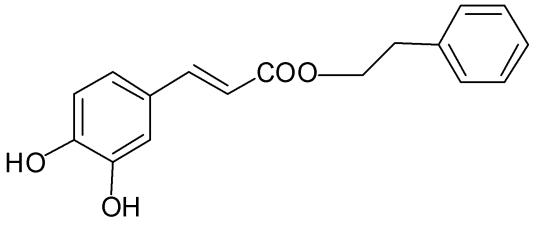


Fig. 1. Caffeic acid phenethyl ester

The simple molecular structure, combined with the authority of the senior author Nakanishi, and the remarkable activity of the compound were the reason for the ever increasing interest of scientists in this compound in the years after 1988. At present, it is the most studied of all the individual constituents of propolis, with over 970 hits for "caffeic acid phenethyl ester" in title in Google Scholar. In the Scopus scientific database, we found 1277 hits for "caffeic acid phenethyl ester" in title, abstract and/or keywords, 517 of them being in the Subject Area "Medicine". In the Reaxys Database, 20 patents were found which mention CAPE, mostly as a constituent of pharmaceutical preparations for treatment and prevention of cancer, for treatment of diabetic wounds, bacterial and viral infections, etc. Different aspects of the biological and pharmacological activities of CAPE have been studied, including the involved mechanisms of action. It was found to possess antibacterial, antiviral, anti-inflammatory, cytotoxic, neuroprotective, hepatoprotective, cardioprotective activity (recent reviews: [2–4]). One of the most important features of CAPE is its ability to specifically inhibit NF-kappa B activation and this may provide the molecular basis for its multiple immunomodulatory and antiinflammatory activities, including induction of apoptosis [5]. Because of the latter property, CAPE is widely used as a standard NF-κB inhibitor in experimental studies [6]. The interest in the versatile and potent pharmacological activities of CAPE led to a significant number of publications dealing with different ways of synthesis of caffeic acid phenethyl ester, last reviewed in 2014 [4]. Natural sources of CAPE and methods of analysis of this important secondary metabolite have not been reviewed yet. That is why the present review summarizes the available literature data on CAPE natural sources, analytical techniques for determination of CAPE in different matrices, and latest development in synthetic approaches (newly published after 2014).

Natural sources of CAPE. The first known and the most well-known natural source of CAPE is the bee product propolis. Propolis is a plant-derived product. To produce it, honeybees collect resinous plant materials, generated by a variety of botanical processes, in different parts of plants. These are substances actively secreted by plants as well as substances exuded from wounds in plants: lipophilic materials on leaves and leaf buds, mucilages, gums, resins, latices, etc. [7]. As a result, propolis chemistry depends on the source plant,

respectively on the specificity of the local flora at the site of collection and the choice of plant resin for propolis production made by bees. In a particular phytogeographic region honeybees demonstrate unambiguous and constant preference to particular plant source(s) and consequently, different propolis chemical types exist [8,9]. It turned out that CAPE can only be found in one of these types: poplar type propolis, which is typical for the Temperate zone. It is a constituent of the resinous exudates on buds and young leaves of different species of *Populus*, mainly *Populus nigra* L. [10–12]. That is why poplar bud resin is, besides propolis, another natural source of CAPE. However, not every *Populus* species bud resin contains CAPE: it was identified in bud exudates of *P. nigra*, *P. angustifolia* [13,14], *P. ciliata* [15], *P. deltoides* [16], *P. tristis* [17], *P. cathayana*, *P. szechuanica* [18], *P. koreana*, *P. maximowiczii* and *P. suaveolens* [19], *P. simonii* and *P. yunnanensis* [20], *P. violascens* [21], *P. canadensis* [22]. Detailed GC-MS analyses failed to detect CAPE in bud exudates of *P. trichocarpa* [23], *P. balsamifera* [24], *P. lasiocarpa* [25], *P. euphratica*, *P. fremontii*, *P. sargentii*, *P. wislizenii* [16], *P. sieboldii* [27], *P. candicans* [28], *P. laurifolia* [29], *P. tremula* [30,31], *P. tremuloides* [32].

It is important to note that CAPE, together with other propolis constituents, is found in low concentrations in honeys from the Temperate Zone [33].

Many research articles dedicated to CAPE's pharmacological properties claim that CAPE is found in a variety of plants. As a matter of fact, the number of plant species reported to contain CAPE is surprisingly small. Except the abovementioned *Populus* species, the list of plants reported to produce CAPE is quite short.

In 1989 CAPE was identified in a plant different than *Populus*: it was detected by GC-MS as a constituent of the leaf exudates of *Baccharis sarothroides* [34]. Soon after that, it was isolated also from the Brazilian plant *Alibertia macrophylla* [35]. Later, CAPE was found in the methanol extract of roots of *Rhodiola sacra* from Korea. It was identified as the component responsible for the potent anti-inflammatory activity of the extract [36]. It was isolated from the Argentinean highland plant *Parastrephia lucida* (Meyen) Cabrera, used in traditional medicine as an antiseptic and anti-inflammatory crude drug [37]. CAPE was found to be one of the antioxidant and antimicrobial constituents of the flower extract of *Melaleuca cajuputi* [38]. The presence of CAPE was detected by HPLC-MS/MS in *Citrullus colocynthis* from Oman, a popular medicinal plant in Arab countries [39]. The most recent communication on the occurrence of CAPE in a plant is the article of Shin et al. [40]. They report the isolation of CAPE from twigs of *Cinnamomum cassia*, widely used as one of the primary ingredients in Chinese/Korean traditional medicines that have anticancer effects.

There are a number of articles claiming the presence of CAPE in some mushrooms. In his review on medicinal mushrooms, Wasser [41] wrote: "For instance, the caffeic acid phenethyl ester (CAPE), which specifically inhibits DNA binding

of NF-κB and has shown some promising results in human breast cancer MCF-7 cells, was found to be produced by *Agaricus bisporus*, *Marasmius oreades*, *Lentulus edodes*, and *Phellinus linteus*." However, none of the references cited as source of this information mentions CAPE. We could not find any publication in Scopus, Web of Science and Google Scholar databases reporting experimental evidence for the presence of CAPE in mushrooms. Even the publication entitled "Separation and purification of the antioxidant compounds, caffeic acid phenethyl ester and caffeic acid from mushrooms by molecularly imprinted polymer" [42] actually reported only the separation and purification of caffeic acid. CAPE was not detected in any of the 25 tested mushroom species. Thus, the presence of CAPE in mushrooms is yet to be confirmed.

New synthetic approaches to caffeic acid phenethyl ester. In general, synthetic efforts are inspired by the fact that isolation and purification of CAPE from natural resources is expensive, low yielding and time consuming. Naturally CAPE coexists with several catechol ring containing phenolics which present a major hurdle for its isolation. Because of the remarkable biological and pharmacological activities of CAPE and the relative simplicity of its molecular structure, the ways to obtain CAPE synthetically began receiving widespread attention. The synthetic methods were summarized in a review article published in 2014 [4]. In general, they could be classified in three groups: methods using caffeic acid as starting material (direct esterification, reaction with phenethyl bromide, the acyl chloride method); methods using 3,4-dihydroxy benzaldehyde as starting material (Wittig reaction, Knoevenagel-Doebner reaction, one pot reaction using Meldrum's acid), and enzyme catalyzed reactions (chlorogenic acid hydrolase catalyzed synthesis, lipase catalyzed synthesis) [4].

After 2014, innovative approaches to CAPE synthesis focused mainly on the application of up-to-date green solvents, such as ionic liquids and natural deep eutectic solvents. Data are summarized in Table 1.

In several publications, ionic liquids (ILs) have been used as reaction media for enzyme-catalyzed processes. Unlike conventional organic solvents used in biocatalytic reactions, ILs are able to dissolve a wide range of polar and nonpolar organic compounds and there have been numerous studies on enzyme-catalyzed reactions in ILs. It was reported that the use of ILs as reaction media enhances the reactivity, selectivity, and stability of enzymes [43]. Using trialkylphosphine oxide as a complexing agent, a novel biphase system of reactive solvent and IL was firstly reported for CAPE production from methyl caffeate and 2-phenylethanol catalyzed by lipase via transesterification [44] (reported yield over 90%). The lipase-catalyzed synthesis of CAPE from caffeic acid and 2-phenylethanol was enhanced by using a novel dimethyl sulfoxide–IL co-solvent [45] (conversion over 96%). To establish a high-efficiency bioprocess for obtaining CAPE, a novel continuous flow enzyme catalyzed (Novozym 435) synthesis of CAPE from alkyl caffeate and 2-phenylethanol (PE) in the IL [Bmim][Tf_2N] using a packed bed

Table 1

New synthetic approaches to CAPE (published after 2014)

Starting materials	Type of reaction	Catalyst	Reaction medium	Yield (%)	Ref.
Enzymatic catalysis					
Methyl caffeate, 2-phenylethanol	Transesterification	Lipase	Biphase system of trialkylphosphine oxide – cyclohexane/ [Bmim][Tf2N]	> 90	[44]
Caffeic acid, 2-phenylethanol	Esterification	Lipase	Co-solvent DMSO– [Bmim][Tf2N]	96	[45]
Alkyl caffeate, 2-phenylethanol	Transesterification	Novozym 435	[Bmim][Tf2N] (continuous flow in packed bed microreactor)	93	[46]
Caffeic acid, 2-phenylethanol	Esterification	Whole cells *A. niger* EXF 4321	[Emim][Tf2N]	80	[47]
Chemical catalysis					
Caffeic acid, 2-phenylethanol	Esterification	Acid (p-toluene-sulfonic, sulphuric, Ambersyt 15)	Choline chloride: caffeic acid 2:1	76	[49]
Caffeic acid, 2-phenylethanol	Esterification	$Yb(OTf)_3$	Nithomethane	74	[50]
Caffeic acid, 2-phenylethanol	Esterification	$[Bmim][BF_6]$	$[Bmim][PF_6]$, microwave	95	[51]
1,2-di-O-acetyl-4-bromocatechol, phenethyl acrylate	Heck reaction	$Pd(OAc)_2$	Toluene-dimethyl formamide	44	[54]

microreactor was developed [46] (yield 93%). ILs were also applied as green reaction media for the esterification of CA and PE using whole cell catalysis by the halotolerant filamentous fungus *A. niger* EXF 4321 [47] (yield 80%).

Natural deep eutectic solvents (NADES) are an alternative to the ionic liquids. NADES are mixtures of solid natural compounds which produce a eutectic mixture with a melting point that is much lower than the one of the individual components and, like ILs, are liquid at ambient temperature (Dai et al. [48]). Recently, a NADES was designed and successfully used as a reaction medium for obtaining CAPE: acid catalyzed reaction of CA and PE was carried out in a NADES made of caffeic acid and choline chloride. Caffeic acid is part of the

solvent and a reactant; phenethyl alcohol and the product CAPE are soluble in this NADES, and no additional solvent is necessary [49] (optimized yield 74%).

Some authors have tried to find alternative chemical catalysts for the esterification process. A fast catalytic method for the preparation of CAPE and other caffeic acid esters was reported: the esterification was carried out in the presence of ytterbium triflate in nitromethane without any other auxiliary reagents [50] (yield about 50%). Microwave assisted ionic liquid catalysis was successfully applied to perform the esterification of caffeic acid with 2-phenylethanol: the ILs acted as both solvent and catalyst in this chemical synthesis [51] (95% conversion). A recent Chinese patent claims the use of microwave-assisted reaction of CA and PE with molecular sieve as catalyst [52].

Recently, an alternative way to CAPE synthesis was developed based on the Heck reaction: arylation and alkenylation of alkenes under the influence of a palladium catalyst, [53]. A total of three steps were operated: protecting reaction, Heck reaction and de-protecting reaction, with 4-bromocatechol and acrylic acid phenethyl ester as starting materials. CAPE was obtained in an overall yield of 40% under the optimized conditions [54].

For this reason, it can be assumed that attempts to synthesize CAPE mainly by enzymatic catalysis and the use of modern "green" solvents will go on in the years to come, encouraged by the high demand for this modest compound with powerful biological activity.

Quantitative analysis of CAPE. ***Analysis in natural sources.*** CAPE is one of the most important biologically active constituents of propolis, and its quantitative analysis has been a challenge to propolis researchers since 1989 (Table 2). Several procedures for quantification of CAPE in propolis extracts were developed, based on GC after silylation with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) [31,55]. Because most biologically active constituents of propolis, including CAPE, are non-volatile and require derivatisation prior to GC analysis, HPLC is much more suitable for quantification of individual propolis constituents. Hyphenated techniques combining liquid chromatography with UV diode array detector (DAD) and mass spectrometry have found wide application in quantitative analysis of CAPE in propolis and propolis extracts: HPLC-UV/DAD [56,57], liquid chromatography-electrospray ionization mass spectrometry (LC-ESIMS) [58], HPLC-electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS/MS) [59], ultra performance liquid chromatography (UPLC) [60], LC-DAD-MS [61]. Only three of the above-mentioned procedures were focused exclusively on CAPE, as an indicator of the quality of propolis [55,57,58]. All the other methods quantify simultaneously a number of propolis phenolics: flavonoids, phenolic acids and their esters including CAPE. Recently, a new method based on near-infrared spectroscopy (NIR) to estimate the amount of CAPE in propolis samples was proposed, which could be used as a quality parameter of in situ raw propolis, without any preprocessing [62].

Table 2

Analytical procedures for quantification of CAPE

Matrix	Sample preparation	Analytical method	Target compound/s	Ref.
Analysis in natural sources				
Propolis	Silylation of dry EtOH extract	GC-MS	Propolis phenolics, incl. CAPE	[31]
Propolis	Silylation of dry EtOH extract	GC-MS	CAPE	[55]
Commercial hydroalcoholic solutions	Dilution with MeOH, filtration	HPLC-DAD	Propolis phenolics, incl. CAPE	[56]
Propolis	Extraction with 80% EtOH	HPLC-DAD	CAPE	[57]
Propolis	Extraction with EtOAc	LC-ESI MS	CAPE	[58]
Propolis	Extraction with MeOH, dilution with water	HPLC-MS/MS	Propolis phenolics, incl. CAPE	[59]
Propolis	Extraction with MeOH	UHPLC	Propolis phenolics, incl. CAPE	[60]
Propolis	Extraction with EtOH	LC–DAD–MS	Propolis phenolics, incl. CAPE	[61]
Propolis	Freezing at −20 °C, pulverization	NIR	CAPE	[62]
Analysis in biological fluids				
Rat plasma	Extraction with EtOAc	HPLC-UV	CAPE, FCAPE (2-fluorocaffeic acid phenethyl ester)	[63]
Rat plasma	Extraction with EtOAc	HPLC-UV	CAPE, CAPA (caffeic acid phenethyl amide)	[64]
Rat plasma	Extraction with EtOAc	LC-ESI MS	CAPE, CAPA	[65]
Rat plasma and urine	Extraction with EtOAc	LC-ESI MS/MS	CAPE	[66]
Dog plasma	Protein precipitation treatment and direct dilution	LC–MS/MS	CAPE, caffeic aicd	[67]

Analysis in biological fluids. The large number of in vivo studies of the physiological and pharmacological effects of CAPE requires analytical methods designed to measure the concentration of CAPE in biological fluids. In this case the matrix is very different from raw propolis and propolis extracts, thus the sample preparation plays an important role. The analytical approaches to CAPE quantification use again liquid chromatography-based hyphenated techniques (Ta-

ble 2). Reverse-phase HPLC methods with UV detection at 320 nm were developed and applied for the stability evaluation of CAPE and its derivatives (caffeic acid phenethyl amide, CAPE fluorinared derivative) in rat plasma [63, 64]. For the quantitative determination of CAPE and caffeic acid phenethyl amide in rat plasma, an LC-ESIMS method was used [65]. LC-MS/MS method was developed and validated for quantification of CAPE in rat plasma and urine, using taxifolin as internal standard [66]. Analysis of CAPE content in dog plasma was achieved by liquid chromatography tandem mass spectrometry (LC–MS/MS) [67]. Sample preparation in most procedures was performed by liquid/liquid extraction with ethyl acetate. In one case [67] the sample pretreatment generally involved protein precipitation treatment and direct dilution.

It can be concluded that reliable, sensitive and selective methods for analysis of CAPE in both natural sources and biological fluids have been successfully developed. Of course, further improvement of analytical approaches could be expected because of the practical importance of the analyte, taking into consideration that pharmacokinetic studies are still scarce.

Conclusions. In recent years, the number of studies dedicated to different aspects of the chemistry and biological importance of caffeic acid phenethyl ester has been steadily growing. Concerning natural sources, the question about the presence of CAPE in mushrooms is debatable and needs further studies to be clarified. In the years after 2014, the attempts to find an efficient, fast, economic, and environmental friendly way to synthesize CAPE focused on the application of new "green solvents" as reaction media, especially for enzyme catalyzed reactions, and further efforts in this field could be expected. Recent analytical endeavors are directed towards new possibilities for CAPE quantification in biological matrices.

Despite the well-known important pharmacological properties of CAPE, it has not yet been investigated as a protective agent or a potential therapy in humans. One of the serious hindrances to clinical trials is its low solubility in water. Finding a way to overcome this obstacle will be one of the future directions in research on CAPE.

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