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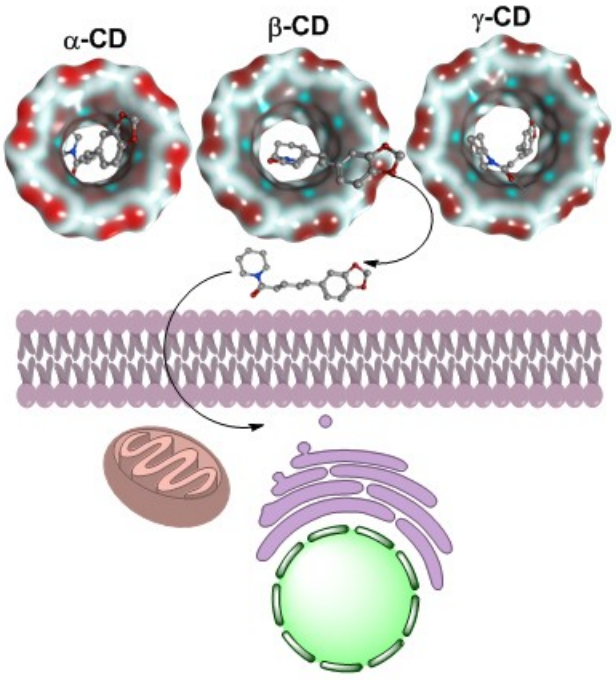

Phytochemical and pharmacological attributes of piperine: A bioactive ingredient of black pepper

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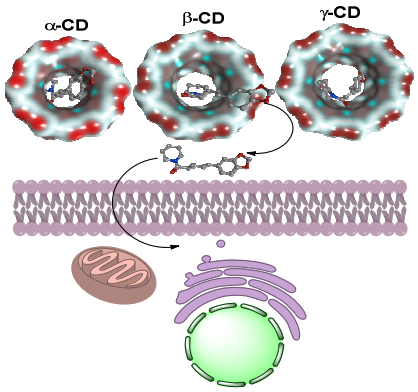
Graphical abstract



Abstract

Plants are vital for the wellbeing of humankind in a variety of ways. Some plant extracts contain antimicrobial properties that can treat different pathogens. Most of the world's population relies on medicinal plants and natural products for their primary health care needs. Therefore, there is a growing interest in natural products, medicinal plants, and traditional medicine along with a desire to design and develop novel plant-based pharmaceuticals. These plant-based pharmaceuticals may address the concerns of reduced efficacy of synthetic antibiotics due to the emergence of drug-resistant pathogens. In this regard, some plant extracts from black pepper (*Piper nigrum*) with antimicrobial properties, including piperine, have the potential to be used as natural dietary supplements together with modern therapeutic approaches. This review highlights possible applications of piperine as the active compound in the fields of rational drug design and discovery, pharmaceutical chemistry, and biomedicine. We discuss different extraction methods and pharmacological effects of the analyzed substance to pave the way for further research strategies and perspectives towards the development of novel herbal products for better healthcare solutions.

Keywords: black pepper; piperine; bio-active compounds; chemical synthesis; extraction; medicinal chemistry; rational drug design; pharmacology

1. Introduction

For as long as humans and animals have existed, they have depended on plants for nourishment and other health benefits. Therefore, there has always been growing attention from the scientific community to plants and their products as additional supplements to synthetic antimicrobials to treat various multidrug-resistant pathogens [1]. Different plant species have been widely used as food flavoring agents, colorant and preservative substances for many centuries across the globe [2]. These plant components were applied in industry and research to extend food shelf-life or to prevent it from spoilage and food-borne diseases [3]. As bioactive plant compounds, they have strong antimicrobial and insecticidal properties widely used in traditional medicine to inhibit or eradicate some infectious pathogens [4]. The well-known antibacterial efficacy of some species, such as black seed (*Nigella sativa*), garlic bulb (*Allium sativum*), thyme (*Thymus vulgaris*), onion (*Allium cepa*), clove (*Syzygium aromaticum*), oregano (*Origanum vulgare*), cinnamon bark (*Cinnamomum verum*), cumin (*Cuminum cyminum*) and many more have been extensively tested and verified [5].

Recent scientific findings, concerning the medicinal applications of bioactive substances from plant extracts, have sparked more interest for further development of novel plant-based pharmaceuticals [6]. This initiative might be very important for more than 80% of the world's population, who are still largely reliant on plant-based medicines and natural products as a primary source of treatment [7]. Additionally, it has been shown by the previous publications that approximately 25% of all medications are derived from plants [8-10].

About 500 various herbal species have been used in modern medicine to treat various illnesses [11] based on anti-inflammatory [12], antioxidant [13] and spasmolytic [14] properties of plant-derived drug-like substances [14].

The last decade has witnessed an unprecedented growth of herbal medicine all over the world [12]. Black pepper, which is widely used in the seasoning, contains bioactive ingredients in its oleoresin fraction, such as essential oils and alkaloid piperine [14]. Moreover, the latter substance can be considered as the main ingredient of black pepper, possessing diuretic and anti-asthmatic effects [14]. As a GIT (Gastrointestinal Tract)-active agent, piperine can facilitate the activation of pancreatic enzymes in the gut [15].

However, piperine has been proven to be only slightly soluble in water [16], restricting its therapeutic effects and biomedical applications. Therefore, this chemical substance should be

administered in high therapeutic doses due to its poor dissolution and gut absorption rates, which might be toxic for the reproductive and central nervous systems [17, 18]. Some attempts have been made to develop novel piperine formulations to enhance its bioavailability, using piperine-encapsulated nanosize liposomes [19], which might be inefficient due to their hydrophobic nature. Therefore, the aim of this review is to give a comprehensive outlook on the phytochemical and phytopharmacological aspects of piperine as an active ingredient, and to discuss future perspectives, considering all the aforementioned effects of piperine important for modern herbal medicine.

2. Piperine applications in traditional medicine

Black paper or *Piper nigrum* is associated with black peppercorns and berries used for seasoning of different dishes. In general, black pepper mainly contains various alkaloids, volatile oils, carbohydrates, starch, and proteins. Being well-known seasoning ingredient, black pepper is known to be a source of an important alkaloid piperine, which adds a strong, pungent flavor to dishes [10, 20].

The usage of black pepper has already been known for many centuries to treat different types of health problems, including intermittent fever, influenza, muscular pain, and migraine [21] in China and India. There is a growing interest from the scientific community in black pepper in general and its alkaloid piperine in particular as a therapeutic agent, stimulating the appetite and the production of saliva [22]. Piperine was also found to increase the orocecal transit time [23, 24] and to act as an anti-tumor agent in mice [25, 26], promoting the enzymatic activity of pancreas and preventing diarrhea [24, 27]. Recently, some studies on the biological properties of piperine have revealed its antioxidant, anticarcinogenic, anti-inflammatory, antiulcer, antithyroid, and antimicrobial effects with some potential to modulate immune responses [28-30]. Additionally, this compound has shown some activity to promote the absorption for some drugs, diminishing their metabolism and cholesterol level in the blood [22, 31].

3. Piperine phytochemistry

Phytochemical analysis of black pepper had shown the presence of various chemicals, including piperine as the first pharmacologically active compound isolated from the Piperaceae family [32]. However, the other chemical substances were also purified subsequently from black

pepper, comprising phenols, flavonoids, alkaloids, amides, steroids, lignans, neolignans, terpenes, chalcones, etc [24]. While some of these compounds, like piperonylamine, pipericide, sarmentosine, sarmentine, chavicine (Figure 1) already identified as bioactive, the other molecules (piperine) were determined to show a significantly higher pharmacological effect [32-38]. In particular, piperine is believed to be the main bioactive chemical component with antimicrobial activities purified from *P. nigrum* [39]. This chemical was first extracted from Piper nigrum in 1819 by Hans Oersted [40]. In the pure form, it represents a yellow crystalline powder of piperonyl-piperidine, reacting as a weak base in the solution [41, 42]. Additionally, piperine has also its cis-trans isomeric structures, comprising the trans-trans (piperine), cis-trans (isopiperine), cis-cis (chavicine), and trans-cis (isochavicine) isomers. Apart from piperine, none of these isomers possess the pungency taste [22]. However, the piperanine, piperettine, piperylin A, piperolein B, and pipericine alkaloids extracted from black paper might maintain some small pungent flavor in the experiment [22, 43].

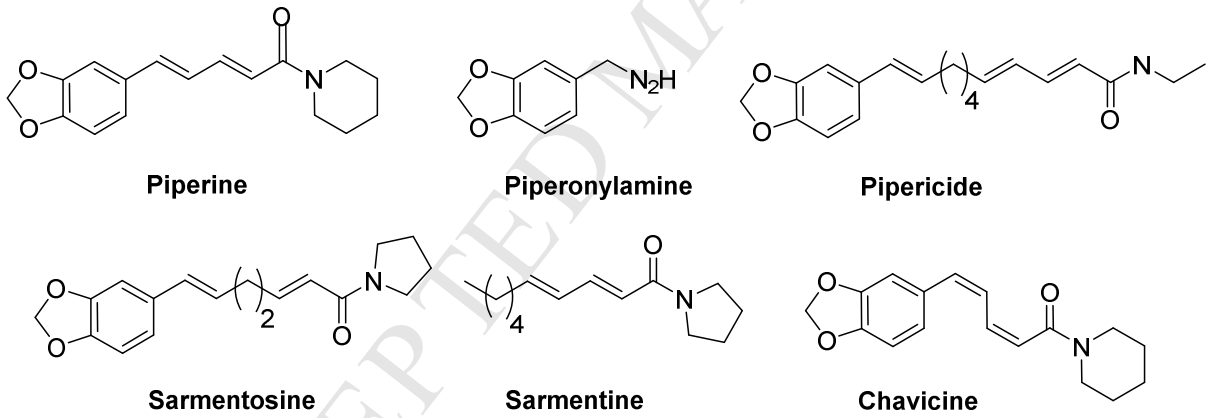


Fig. 1. Some chemical substances derived from *P. nigrum* (Adopted from Ref. Ahmad et al., 2012 with modifications [26]).

4. Antimicrobial effects of black pepper

Apart from being used as a seasoning ingredient, black pepper could be applied as an antimicrobial agent against various antibiotic-resistant pathogens in addition to the conventional medication (Table 1).

Table 1 Antimicrobial activities of piperine against different micropathogens (Adopted from Ref. Aldaly et al., 2010 with modifications [39])

Tested microorganisms	Piperine 100 mg/disc	Streptomycin 10 µg/disc	Erythromycin 15 µg/disc	MIC (mg/ml)
E. coli	20	13.5	15	6.25
Staphylococcus aureus	12	23	24	50
Klebsilla pneumonia	15	20	10	25
Proteus vulgaris	17	10	6	12.5
Pseudomonas aeruginosa	8	22	18	100
Candida albicans	23	N/A	N/A	3.125

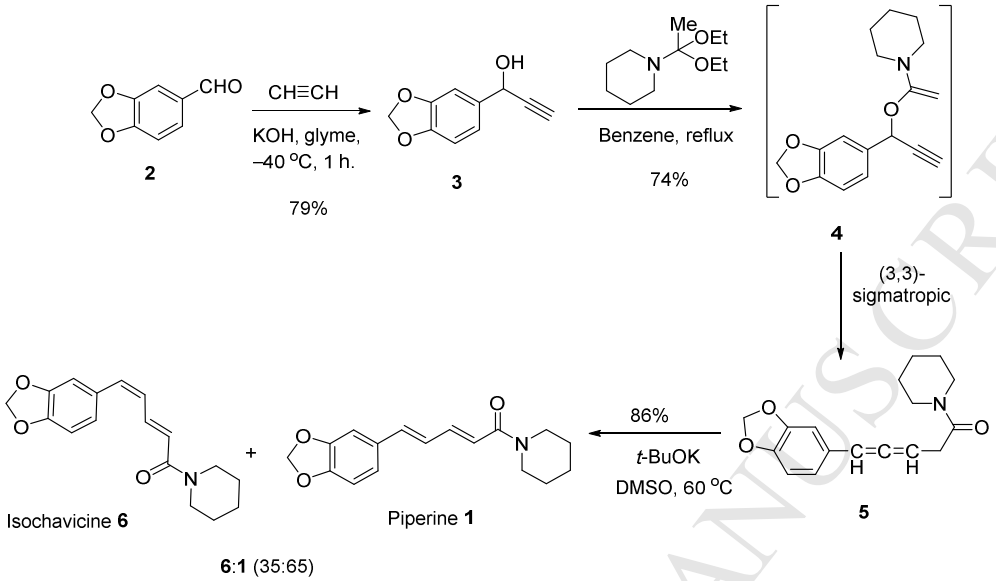
MIC: minimum inhibitory concentration

It was determined to be most effective against the pathogenic Gram-positive strains as *Staphylococcus aureus, Bacillus cereus*, and *Streptococcus faecalis* [4]. On the other hand, the Gram-negative bacteria (*Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli*) are known to be less susceptible to black pepper [44]. Moreover, the aqueous extracts of black pepper might possess the permeability through lipid membranes of Gram-positive microbes at the concentration of 10 µl/disc to already exhibit the antimicrobial effect [45, 46]. Some studies have been conducted to investigate the antimicrobial and antifungal activity of different alkaloids extracted from black pepper, including tannins, flavonoids, and glycosides [47, 48]. Furthermore, the black pepper extracts can be formulated with metal-contained nanoparticles to protect the agricultural crops from plant pathogens [49].

5. Piperine synthesis

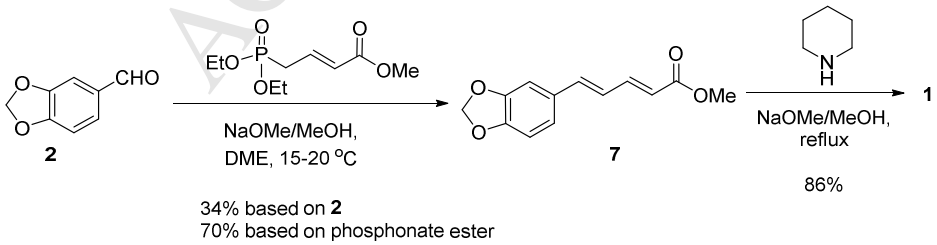
Many synthetic strategies for the synthesis of piperine in literature have been reported, but six of them will be discussed [50-55]. One of the earlier reports about piperine synthesis is Tsuboi and Takeda strategy in 1979 [50]. They described this synthesis in three steps starting from cheaply and commercially available aldehyde called piperonal (**2**) (Scheme 2). The addition of piperonal (**2**) to acetylene suspension in the presence of base like KOH at –40 °C afforded the propargylic alcohol **3** in 79% yield. The propargylic alcohol **3** was then subjected to thermal condensation with *N*-acetylpiperidine diethyl acetal to give intermediate **4** which then undergo (3,3)-

sigmatropic rearrangement to release allene amide (**5**) in 74% yield. The allene amide **5** was converted to a mixture of piperine (**1)** and isochavicine (**6**) with 65:35 ratio in the presence of *t*-BuOK within overall yield of 86%.



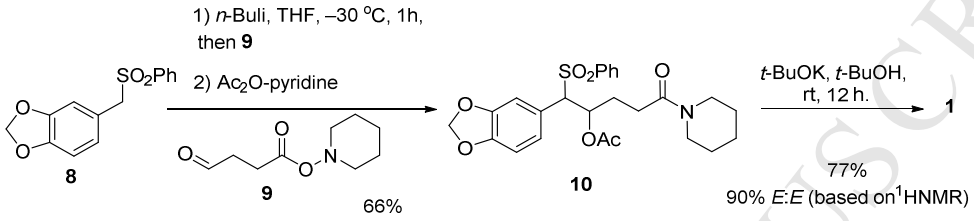
Scheme. 2. Tsuboi and Takeda strategy for synthesis of piperine (**1**).

Two years after Tsuboi and Takeda synthetic pathway, Olsen and Spessard published a two-step synthesis of piperine with an efficient stereoselective control of the two double bonds (Scheme 3) [51]. Their two-step approach involved a vinylogous Wadsworth-Horner modified Wittig condensation of piperonal with the anion derived from methyl (*E*)-4-diethylphosphono-2-butenoate to give methyl piperate (**7**) with 34% yield based on peiperonal (**2**) and 70% based on phosphonate ester. This transformation is an excellent method to yield two *trans* alkenes, although the yield was not high based on piperonal. Piperine then was obtained with 86% yield according to the methoxide-catalyzed aminolysis of methyl piperate with piperidine.



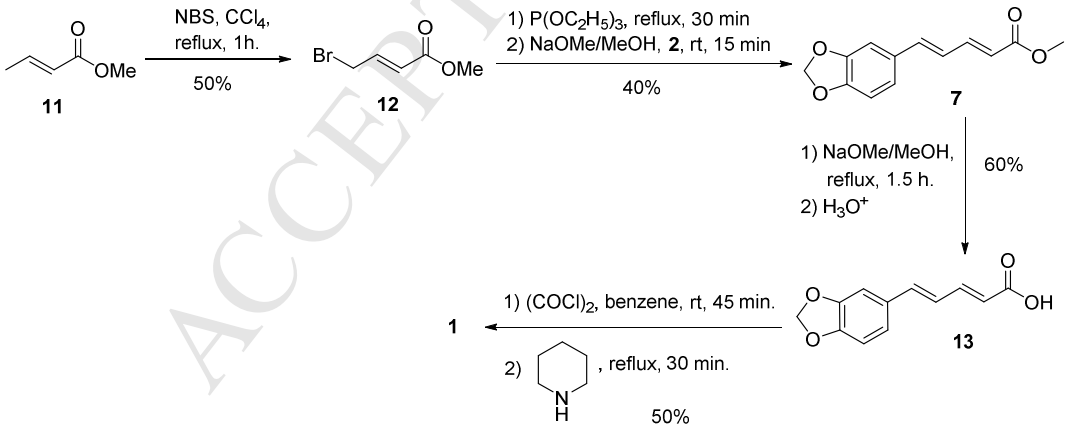
Scheme. 3. Olsen and Spessard synthetic strategy of piperine (**1**).

In 1986, Mandai *et al.* documented also two-step synthesis of piperine [52]. They reported a highly stereoselective synthesis of piperine through a double elimination reaction of *β*-acetoxy sulphone (Scheme 4). Their strategy involved coupling of sulphone **8**, which was synthesized from piperonal (**2**), with aldehyde **9** in the presence of a strong base *n*-BuLi to give acetate **10** in 66% yield. Double elimination of acetate **10** using *t*-BuOK yielded piperine in 77% yield with good stereocontrol of 90% *E*:*E*.



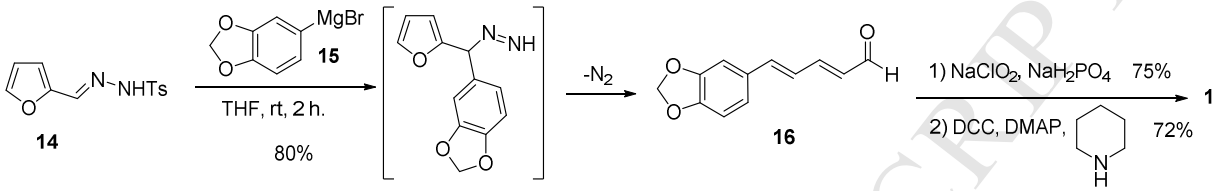
Scheme. 4. Mandai *et al.* synthetic strategy of piperine (**1**).

In 1995, Sloop reported a microscale synthesis of piperine involving transformation of methyl crotonate (**11**) into ester **13** (Scheme 5) [53]. This access through allylic bromination of methyl crotonate by N-bromosuccinimide (50% yield) followed by aldol like condensation to yield methyl piperate **7** which with a moderate yield of 40%. Hydrolysis of methyl piperate **7** followed by aminolysis with piperidine gave piperine with 55% yield over two steps.



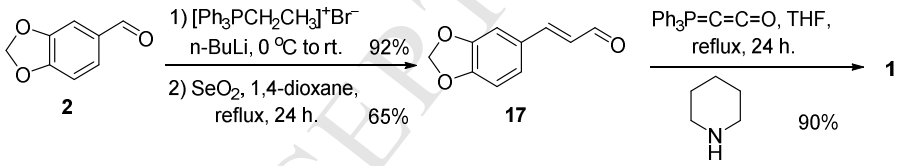
Scheme. 5. Sloop synthetic strategy of piperine (**1**) from methyl crotonate.

In 2000, Chandrasekhar *et al.* reported a successful synthetic strategy through the formation of dienal **16** (Scheme 6) [54]. The dienal **16** was obtained via the addition of Grignard reagent of piperanol **15** to aldehyde tosylhydrazones in 80% yield. Pennick oxidation of dienal led to piperic acid **13** followed by aminolysis in 73% yield overall two steps.



Scheme. 6. Chandrasekhar *et al* strategy to the synthesis of piperine (**1**).

Finally, Schobert *et al*. in 2001 reported an intermolecular three-component reaction between aldehydes, amines and ketenylidenetriphenylphosphorane ($Ph_3P=C=C=O$) lead to a selective formation of piperine (Scheme 7) [55]. Their strategy started from conversion of piperanol into α,β-unsaturated aldehyde **17** in two steps; olefination with ethylidenetriphenylphosphorane to give *cis–trans*-isomeric mixtures of 3,4-(methylenedioxy)-β-methylstyrene, and a *trans*-selective allylic oxidation with selenium dioxide to furnish the *E*-aldehyde **17**. The aldehyde 17 was then subjected to the three-component domino reaction with ketene ($Ph_3P=C=C=O$) and piperidine to furnish piperine in 90% yield.



Scheme. 7. Schobert *et al.* synthetic method piperine (**1**) via three-component strategy.

6. Piperine extraction

The piperine compound can be extracted from black pepper in the range of 6-13% by means of organic solvents [56]. Several types of the volatile organic solvents have been used so far for this purpose, comprising acetone, dichloromethane, ethanol, and diethyl ether under specific pressure and time conditions [22]. The piperine purification process depends on various parameters, such as the type of solvent used and the degree of maturation stage of black pepper [57]. Some

alcohol-based solvents tend to be hydrotropic and ionic chemical solutions [14], providing the rapid and cheap extraction of piperine [58]. Almost 95-98% purity of the piperine extract is required to be used in the pharmaceutical industry, and the additional purification might be needed for this by the oleoresin extract [59]. There are some common techniques, which are used for piperine extraction, such as maceration, solvent extraction, and soaking.

All these extraction methods require high temperature and the time-consuming with the high risk of the final product degradation [60]. Some commons mistakes in the extraction technique might include the improper selection of the method and the excessive usage of solvents extra usage of organic solvent [60]. In fact, the microwave- or ultrasound-assisted, and supercritical fluid extraction methods were developed and optimized to enhance the extraction yield of chemical substances. Therefore, the modern extraction techniques summarized in Table 2 are discussed in more detail in the next sections.

Table 2 Different extraction techniques used to extract piperine

Extraction technique	Extraction time	Extraction yield (w/w)	Benefits	Disadvantages	Reference
Ultrasound assisted extraction (UAE)	18 min	0.58%	Short running time, higher extractive yield, controllable parameters	Small particle size, more filtration steps	[61]
Microwave assisted extraction (MAE)	2 min	94%	Selective, short running time, high extraction	More filtration steps, time consuming during cooling	[62]
Double bypass Soxhlet apparatus (DBSA)	12 ± 1 h	3.90% ± 0.10%	Easily operate, simple	Long extraction time, solvent consuming	[63]
Hydrotropic solubilization	2 h	90% to 96%*	Minor purification steps, unlike surfactant not foaming	-	[62]

Supercritical fluid extraction (SFE)	2 to 5 h	6.7% to 7.6%	Efficient, selective, clean, fast	High cost, less pressure resistant	[64, 65]
Ionic liquid ultrasound assisted extraction (IL-UAE)	30 min	3.57%	Environment friendly, Short extraction run, high efficiency,	-	[66]
	18 min	1.96%			

241

242

243 *6.1. Soxhlet extraction*

244 The Soxhlet extraction technique has been used in the past for the extraction of biologically
245 active compounds [67]. However, this methodology might be considered outdated and not very
246 efficient in comparison to more advanced extraction procedures [68].

247 The performance of this method and its modifications to extract piperine from black pepper has
248 been evaluated by Subramanian and coauthors, showing that the DBSA (Double By-Pass Soxhlet
249 Apparatus) approach outperforms the other techniques in terms of the decreased extraction time
250 due to the overall increase of extraction cycles [63]. The extraction results showed the improved
251 extraction yield in 3.9% from DBSA after 12 hours of extraction [63]. In the study of
252 Rajopadhye and coauthors, the black pepper roots were used for the Soxhlet extraction with
253 methanol, obtaining the peperine concentration of 9.56 ± 0.83 mg/g [69]. The other authors
254 applied the supercritical fluid (CO_2) extraction together with the Soxhlet method to extract
255 piperine from the corn, reaching the maximal piperine concentration (56.6 mg/g) by using the
256 former methodology [69].

257

258 *6.2. Hydrotropic extraction*

259 Various hydrophobic molecules can be extracted from disrupted plant cells by using the
260 hydrotropic solutions [70]. The hydrotropic solutions of *Piper nigrum* plant extracts form
261 permeable component, allowing the adsorption of hydrophobic molecules, such as piperine, on
262 cellulose during the extraction process [71]. The whole process can be controlled by changing
263 some parameters, like particle size, temperature, and the amount of hydrotropic solvent [70]. A
264 relationship was observed between the extraction efficiency and the alkyl chain length of the

hydrotrope [72]. In particular, some hydrotropic solvents, including sodium p-toluene sulfonate, sodium xylene sulfonate, sodium-butyl benzene sulfonate, and sodium cumene sulfonate were already tested to extract piperine [71]. The latter molecule had shown the best performance in this process in comparison to the other solvents due to its longest chemical chain [71].

The other factor, such as the hydrophobic volume of a molecule also influences the extraction process, following a similar pattern [72]. Additionally, it was determined that the extraction process of piperine is optimal at 30°C, using this particular extraction technique [71]. This condition was considered to be most effective for the piperine transport into the hydrotropic medium and finally to on cellulose.

The particle size reduction of a substrate from 710 to 50 μm might also directly interfere with the purity of the extracted piperine. As per reduced particle size, the cellular disintegration would be increased so as the efficiency of hydrotropic solution leaching into the cellular matrix. This process benefits the purity of extracted piperine from 89 to 98% [71].

6.3. Supercritical fluid extraction

The supercritical fluid extraction (SFE) method becomes a very popular technique for the extraction of different drug-like molecular compounds from various sources, including plants [73]. Moreover, it is mainly considered as the clean, efficient, selective and rapid extraction process [73]. The SFE methodology implements different solvents with high molecular densities to archive more efficient compound extraction [73]. In fact, the SFE mechanism implies the effective mass transfer via fluids with much greater molecular diffusion and smaller viscosity than other extraction techniques [73]. On the other hand, this method is using the temperature and pressure for liquid carbon dioxide in the range of 31.1 °C and 73.8 bar, [74] with low polarity level, which plays a significant role in the extraction of non-polar compounds [73]. To extract the polar compounds, some chemical polar substances were used as additives to increase the polarity of the mixture in a range of up to 10% of the main supercritical fluid [74].

This method was extensively applied for the peperine extraction in the first decade of the 20th century [75]. The piperine yield was obtained from this extraction to be in the range from 81% and 98% [76], using the pressure of 350 atm and the temperature of 60° [77]. Another SFE extraction protocol developed by Kurzhals and Hubert (1980), using a mixture of propane and carbon dioxide at 52°C and 78 bar, secured the piperine extraction yield up to 98% [78].

Furthermore, Sovova et al. (1995) have also performed the SFE extraction experiments with the same parameters, resulting in the piperine extraction up to 7.6% by its weight [64].

6.4. Ultrasound-assisted extraction

The ultrasound-assisted extraction (UAE) technique is primarily based on the principles of thermal effects and cavitation, which mediate the mass transport phenomena across different types of cell membranes [79]. In particular, the cavitation bubbles collapse becomes the cause of micro-jets formation and disruption of cells due to the asymmetrical imploding of these bubbles near to the solid surface [80], occurring at high temperature (up to 5000 K) and pressure (1000 bar). The thermo-physical effects produced by this process might create the cellular membrane disruption and the impairment of circulating liquids in the cells [81]. All this increases the UAE extraction yield through the more efficient permeation of the solvents into the plant cells [82]. Overall, the advantages of this method include effective solvent permeation rate and low extraction time and temperature [83]. On the other hand, the UAE extraction also depends on the type of the solvent, the number of extraction cycles, temperature, ultrasound intensity and the solid-solvent ratio [80]. The technique was effectively allied to the extraction of piperine from *Piper longum* by using different organic solvents, such as ethanol, hexane, and acetone [61]. In this study, acetone was found the most effective organic solvent to extract piperine as this extraction is dependent on the polarity index of solvent [61].

6.5. Ionic liquid extraction

The ionic liquid (IL) extraction is a combination of cations and anions of molten salts with the melting point typically below 100°C [84]. The IL technique has some advantages, which makes it the method of choice due to its more stable extraction of various chemicals, using highly polar solvents and low vapor pressure [85].

The physicochemical properties of ionic liquid have a significant impact on the analyte and its extraction efficiencies [66]. These properties are usually correlated with ionic interactions [86]. It is also worth mentioning that the hydrophobic interactions are playing an important role between bio-active compounds and aqueous ILs, hydrophobic interaction in this extraction process, as it was detected for the IL extraction of piperine, tannin, rutin, quercetin, and curcumin [87, 88]. However, the IL approach is usually combined with other extraction methods, such as UAE, to

achieve more efficient extraction yield [66]. For instance, the ionic liquids-ultrasound based extraction (IL-UAE) was devised to enhance the extraction output and to reduce the extraction time [89]. In fact, IL-UAE was utilized to extract piperine from black pepper using four different anions (BF^{-4}, BF^{-}, H_2PO^{-4}, and PF^{-6}) with 1-butyl-3-methylimidazolium (C_4MIM) ionic liquid [66]. The piperine extraction efficiency was dependent on the ionic composition in a descending order in terms of their hydrophilicity as $BF^{-4}>Br^{-}>H_2PO^{-4}>PF^{-6}$ [66]. Finally, the BF^{-4} ionic form with C_4MIM had provided the optimal extraction condition, including ultrasonic power, extraction time, the solid-to-liquid ratio for the piperine purification. In particular, by using these reagents at a concentration of 0.2 M with a solid-to-liquid ratio of 1:15 and an ultrasound power of 500 W, the piperine extraction yield of 3.577% was obtained [66].

6.6. Microwave-assisted extraction

The microwave-assisted extraction (MAE) has been widely implemented to extract various chemical compounds [90]. This technology utilizes the microwave energy, which is absorbed by chemicals in order to evaporate them from the solid raw material. Finally, the condensation of these volatile compounds occurs as the recovering process [91]. MAE can be considered as selective methods that favor polar molecules and solvents with high dielectric constant, producing a heat during the extraction [92]. This heating process is largely generated by microwaves via the ionic induction or dipole rotation [93]. The hydration or soaking phase of extracted material in water plays an important role to control the extraction rate. Some other factors, like the extraction temperature and microwave intensity, have also contributed to the extraction process [90]. At the high microwave intensity, some cellular agglomeration occurs at the beginning of the extraction phase followed by the rapid cellular disruption [94]. In particular, as the power of microwaves increases the extraction rate goes high until the optimum extraction yield is reached [94]. Additionally, the microwave irradiation strength was found to be directly proportional to the solvent loss during the extraction [71]. For instance, when the microwave intensity is increased from 300 to 450 W, the solvent consumption is also elevated from 16 to 20%. For instance, when microwave concentration in the range of 300 to 450 W the solvent loss 16% to 20%. But it decreases when the power of microwaves reduces as 150 W lost the solvent up to 8%. Furthermore, the surface tension and viscosity could also contribute to the solvent loss during the extraction process [95, 96].

The MAE technique was successfully used for the piperine extraction from *Piper nigrum*, where the plant cells had experienced a dielectric heating [71]. During this extraction, the polar and non-polar solvents were used, such as toluene, petroleum ether, heptane, dichloromethane, and ethanol [22]. As a result, the highest extraction was achieved by applying non-polar petroleum ether to intensify the piperine purity from 85 to 94% [22]. On the other hand, the other semi-polar and polar solvents (dichloromethane and ethanol) provided the extraction rate from 75 to 80%, respectively.

7. Piperine detection

Several analytical techniques and quantitative methods (Table 3), including high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), colorimetric assays, Kjeldahl method, and ultraviolet-visible spectrophotometry (UV-Vis), are the most common approaches used in the piperine detection after its extraction from white/black pepper [61, 97].

The Kjeldahl method or Kjeldahl digestion was applied among the first techniques to measure piperine indirectly via evaluation of the total nitrogen amount in black pepper [98]. Previously, this analytical technique was developed for the quantitative determination of nitrogen contained in various organic substances [56]. Before that, the hydrolysis of piperine methylenedioxy group by chromotropic acid was needed for some old colorimetric assays [99]. On the other hand, the UV-Vis method is also a powerful technique to detect the UV absorption spectra of piperine at 343 nm wavelength [100] and to select this compound for its isomers [101]. Additionally, the GC-MS methodology might evaluate the degradation state of piperine while identifying some alkaloids (oleoresin), which are present in black pepper [102]. Presently, HPLC becomes a method of choice for piperine detection with much higher precision capabilities compared to UV-Vis [103]. Moreover, the high-performance thin layer chromatography (HPTLC) as a modification of thin layer chromatography has also been implemented for the detection of piperine from herbal products to provide the most accurate results in the experiment [104]. Finally, the chemical characterization of piperine and its isomeres was also achieved by ^{1}H nuclear magnetic resonance (NMR) spectroscopy [105]. In this study, the difference in the coupling constants for the olefinic protons (cis-2 J(H,H)≈11 Hz, trans-2 J(H,H)≈15 Hz) made it possible to determine the configuration of the isolated compounds [105].

Table 3 Analytical techniques used for identification/detection of piperine

Technique	Methodology	Detection parameters	Reference
Colorimetric	Komarowsky method	Piperine heated with defined reagents, purple color develops, absorbance at 570 nm	[99]
	Phosphoric acid method	Piperine heated at 100°C for 8 min, bluish green color develops, absorbance at 635 nm	[106]
	Nitric acid method	Concentrated piperine, alkali and thiourea added, color changes, absorbance at 490 nm	[107]
Gas chromatography-mass spectrometry (GC-MS)	Apolar column BP1	FID (Injector and Detector 300°C)	[102]
UV spectrophotometry	Piperine dissolved in organic solvents and absorbance measured at 343 nm	-	[97]
High-performance liquid chromatography (HPLC)	C18 Mobile Phases; Acetonitrile: water (90:10)	Mobile Phase; Acetonitrile: Water (90:10) at UV 343 nm and 1.5 ml/min flow rate	[61]
High performance thin liquid chromatography (HPTLC)		Mobile Phase; Benzene: ethyl acetate: diethyl ether (60:30:10) at UV 343 nm and 01 ml/min flow rate	[104]
Nuclear magnetic resonance (NMR) spectroscopy	Piperine isomers dissolved in deuterochloroform ($CDCl_3$) to record ^1H-NMR spectra	Addition of tetramethylsilane (TMS) not required	[105]

8. Pharmacological effects of piperine

8.1. Antioxidant activity

It is well-known that various spices and herbs, including a black piper, contain numerous active ingredients, like flavonoids, terpenoids, phytoestrogens and minerals [26]. Among them, piperine was detected to have an antioxidant potential, which might diminish oxidative stress in the cells caused by the high-fat diet [108]. Moreover, piperine was also shown to decrease the level of the thiobarbituric acid reactive substances via the maintenance of catalase, glutathione, glutathione peroxidase, Glutathione-S-transferase, and superoxide dismutase concentrations [108]. This substance could also improve the activity of biotransformation enzymes in the liver in a dose-

dependent way [109]. Furthermore, several studies on the antioxidant activity of piperine have been conducted to establish the reduction of lung metastatic incidence in the B16F-10 melanoma cells through the alteration in lipid peroxidation and the stimulation of antioxidant enzymes [25, 110, 111].

8.2. Anti-inflammatory activity

Various anti-inflammatory effects of substances extracted from plants are known for many therapeutic applications in modern medicine and pharmacy to treat different disease [112]. In particular, some ethanolic and hexane extracts of black pepper have exposed a significant anti-inflammatory activity in mice and rats, using different dosage protocols [113]. Moreover, peperine had also revealed the same activity in the interleukin (IL) 1β-activated fibroblast-like synoviocytes [114], inhibiting the LPS-stimulated endotoxins [115]. Further, piperine might be viewed as a potent immunomodulator, inhibiting airway inflammation a murine model of asthma by the enhanced expression of TGF-beta gene in the lungs [116]. Piperine was also detected to reduce the production of IL-6, MMP-13, and prostaglandin E at the concentration range of 10-100 μg/ml [114]. In another study, piperine was coadministered with curcumin from *Curcuma longa* to suppress a high fat diet-induced inflammation in the C57BL/6 mice and for the prevention of metabolic syndrome [117]. Apart from that, the piperine anti-inflammatory potential had been investigated at colorectal sites, inhibiting the FFA-induced TLR4 mediated inflammation and acetic acid-induced ulcerative colitis in mice [118]. Finally, this compound was evaluated in the carrageenan-induced inflammation assay in mice to assess the analgesic and antiinflammatory activities of piperine activities at the oral dose of 6 mg/kg/day [119].

8.3. Anti-cancer and hepatoprotective activity

The anti-tumor activity of piperine has been detected after its oral administration to reduce the incidence of some forms of gastrointestinal cancers [120]. An alcoholic extract of black pepper, containing piperine, was found to be effective against lung cancer via altering lipid peroxidation, which leads to the spread of free radical reactions and cellular damage [26]. Besides, piperine might restrict the cell cycle at G1/S phase, inhibiting the HUVECs (human umbilical vein endothelial cells) proliferation and migration [121]. In animal models, piperine can hinder angiogenesis, suppressing the tubule formation by endothelial cells and the phosphorylation of

protein kinase B [121]. Some anti-cancer activity of piperine can be seen by applying it in the combination with the FDA-approved antineoplastic compound docetaxel to treat castrate-resistant prostate cancer [122]. By restricting the enzymatic activity of hepatic CYP3A4, piperine decreases the metabolizing rate of this drug in the liver [122]. Additionally, it has also been studied that the application of piperine in a nutritional supplement might also enhance the docetaxel immunosuppressive effects in xenograft animal models without severe side-effects [122]. Piperine was also found to be active against both androgen-dependent and independent prostate cancer cell lines (LNCaP, 22RV1, PC-3, and DU-145), inducing apoptosis through the activation of PARP-1 and caspase-3 proteins [115]. In the LNCaP prostate cancer cells, piperine disrupts the androgen receptor expression, significantly reducing the detection of the prostate-specific antigen [123].

It was previously established that the methanolic extract of black pepper has the hepatoprotective properties confirmed in Wistar rats with induced hepatic damage caused by ethanol- CCl4 [124]. In these experiments, ethanol-CCl4 was administered to increase the levels of triglycerides, alanine transaminase, aspartate transaminase, alkaline phosphatase, and bilirubin. All these parameters came to normal after the animals were treated with the methanolic extract of black pepper [124]. This extract reduced the lipid peroxidation as a hepatoprotective effect at the administered doses alone [125] or in combination with some antituberculosis drugs [125]. In another study, the d-galactosamine-induced liver injury modeled in mice was treated with piperine to normalize the concentration of glutamic oxaloacetic transaminase and pyruvic transaminase levels in serum. The proposed mechanism had been found to be associated with the reduced sensitivity of hepatocytes to TNF-α [126].

8.4. Antidiarrheal, antidepressant, and analgesic activity

The aqueous extract of black pepper was also assessed for its antidiarrheal via promoting the antimotility and antisecretory effects in the gut at a dose of 75, 150, and 300 mg/kg due to the presence of alkaloids (piperine) and carbohydrates [127]. On the other hand, in corticosterone-induced mice model of depression, piperine was examined for its possible antidepressant effect [128]. The depression in animals was evaluated via a decrease of sucrose utilization and an increment of immobility time in the tail suspension test and forced swim test. As a result, in the hippocampus of corticosterone-treated mice, levels of brain-derived neurotrophic factor protein

were significantly reduced in the hippocampus of corticosterone-treated mice [128]. Finally, the piperine treatment of the behavioral and biochemical changes in mice induced by corticosterone had reverted to normal [128].

Furthermore, the acetic acid-induced twitching and tail-flick tests had shown models had shown the prevention of acetic acid-induced writhing in mice after the intraperitoneal (i.p.) administration of piperine at a dose of 30-70 mg/kg in comparison to indomethacin (20 mg/kg, i.p.) [129]. Similarly, the i.p. injections at a dose of 30 and 50 mg/kg for piperine and at a dose of 5 mg/kg for morphine, had significantly increased the reaction time of mice in the tail-flick assay. The analgesic effects of both substances were abolished by the pretreatment of animals with naloxone (5 mg/kg i.p.), suggesting the involvement of the opioid pathway in this process [129].

8.5. Immuno-modulatory activity, bioavailability and cancerogenic effects

The immuno-modulatory activity of piperine was also been examined at a dose of 50 to 250 μg/ml to be cytotoxic for Dalton's lymphoma ascites, Ehrlich ascites carcinoma and L929 cells [25]. In the BALB/c mice, piperine administration caused the increment in total white blood cells, bone marrow cells, and alpha-esterase positive cells [25].

In a murine model of *Mycobacterium tuberculosis* infection, piperine was evaluated to enhance the efficacy of rifampicin [130]. To examine the *in-vitro* immunomodulation of piperine, the mouse splenocytes were used to produce cytokines together with the activation of macrophage and proliferation of lymphocyte. As a result, the piperine-treated splenocytes have shown the enhanced secretion of Th-1 cytokines, improved macrophage activation, and proliferation of B and T cells [130]. To inhibit antigen-induced allergic reactions that control degranulation, piperine can interfere with the IgE-mediated degranulation and cytokine production by RBL-2H3 cells [131].

Some molecular mechanisms underlying piperine activities include a change in the membrane dynamics accompanied by the initiation of protein synthesis linked to the cytoskeleton functioning. This stimulates the passive absorption in the small intestine, thus, supporting the effective drug permeation through the epithelial barriers [132]. However, piperine exhibits poor bioavailability [22] that can be enhanced in situ intestinal absorption models by formulating it with ethyl oleate, Tween 80, and Transcutol P as a self-emulsifying drug delivery system [22].

Additionally, piperine amended the bioavailability of some antibiotics, like ampicillin, norfloxacin [133], amoxicillin, and cefotaxime sodium [134] and herbal compounds (curcumin and resveratrol) via its inhibitory effect to the liver enzymes [135]. However, some studies indicated the adverse effects of piperine on cells because of the 3,4-methylenedioxybenzene moiety presented in the molecule, acting as a carcinogen [136, 137]. Due to this, the piperine structure resembles some other cancerogenic compounds, comprising safrole, methyl eugenol, and estragole [138]. Besides, treatment of cancer cells with piperine provided diminished expression of phosphorylated STAT-3 and NF-kB transcription factors together with a reduction of androgen-dependent and androgen-independent tumor growth [123, 139]. Piperine could be also administered as an effective antitumor agent against lung cancer via activation of caspase-3 and caspase-9 cascades and induction of apoptosis [140].

9. Future perspectives and conclusion

Piperine is a bioactive compound with a broad spectrum of therapeutic activities, which can be extracted from black pepper given this plant its pungent test. Despite the various therapeutic properties of piperine, its biomedical applications are still limited due to its poor bioavailability and low aqueous solubility. This situation can be improved by piperine supramolecular formulation with some hydrophilic substances, including unmodified cyclodextrin (CD) excipients (Figure 2) [141,142]. Recent investigations on the physicochemical properties and solubility of piperine complexes with α-, β-, and γ-CDs (Figure 3) has defined that the CDs interact with the methylenedioxyphenyl group of piperine in a molar ratio of 1:1, influencing the complex solubility [141, 142].

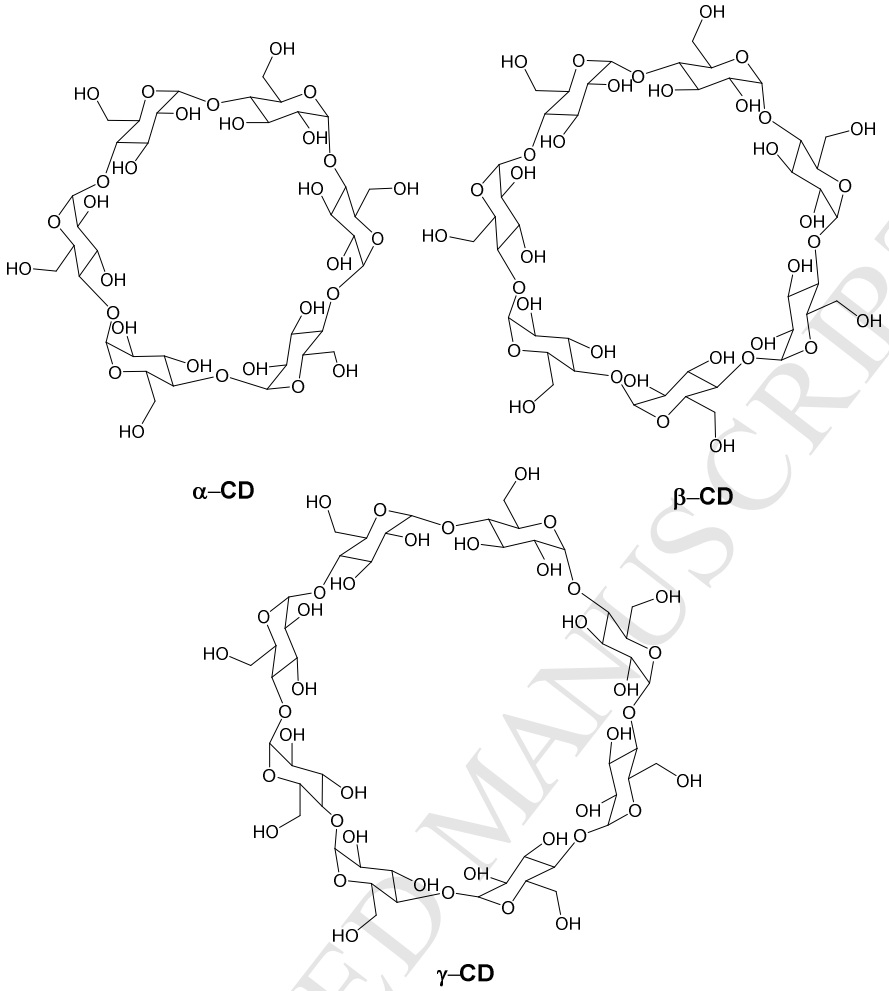


Fig. 2. Chemical structure of unmodified cyclodextrins. The high-resolution graphics were prepared using the ChemDraw software [143].

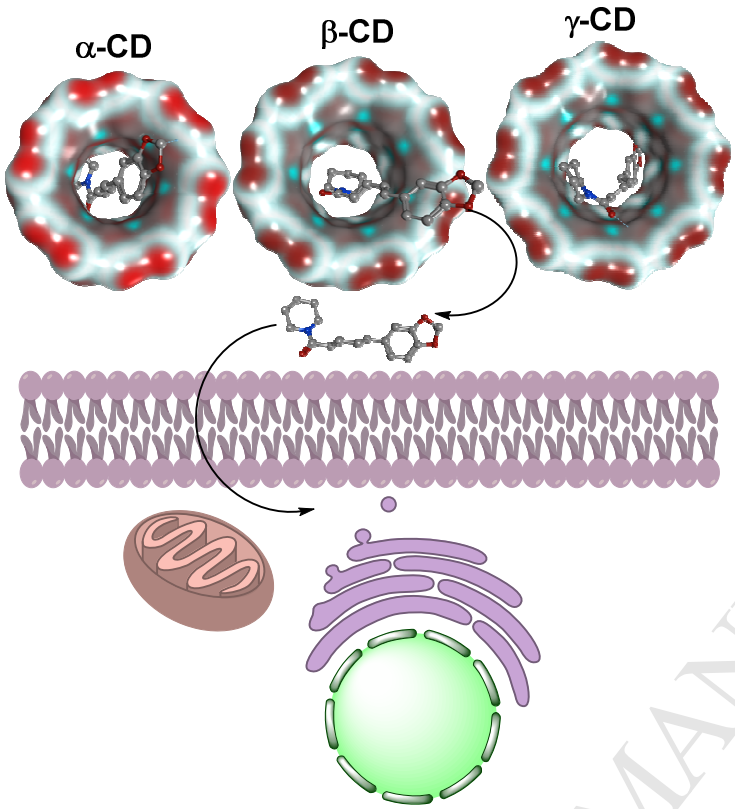


Fig. 3. Piperine formulations with unmodified hydrophilic cyclodextrins (α-, β-, and γ-CDs) shown as hypothetical scheme to improve its aqueous solubility and absorption in the gut after subsequent dissociation of the inclusion complex. The high-resolution graphics were prepared using the ChemDraw and AutoDock software [143, 144].

On the other hand, the quantitative structure-activity relationship (QSAR) paradigm might be applied as a concept where the structural property of drug-like molecules is correlated with their biological activity. It is important to quantify a biological activity in the experiments to match it to the chemical characteristics of drugs, using computational modeling. In particular, this technique has already been used in different biomedical applications to investigate and screen various chemical substances [145-149]. Additionally, the QSAR analysis was applied to the alkaloid piperine to study its pharmacokinetics with respect to the P-gp-mediated multidrug resistance (Figure 4 [A]) and drug metabolism by the P450 3A4 cytochrome (Figure 4 [B]) computationally [150, 151]. Recently, another computational study associated with molecular docking method was conducted to discover novel piperine-derived ligands for the P-gp effective inhibition in bacteria [152].

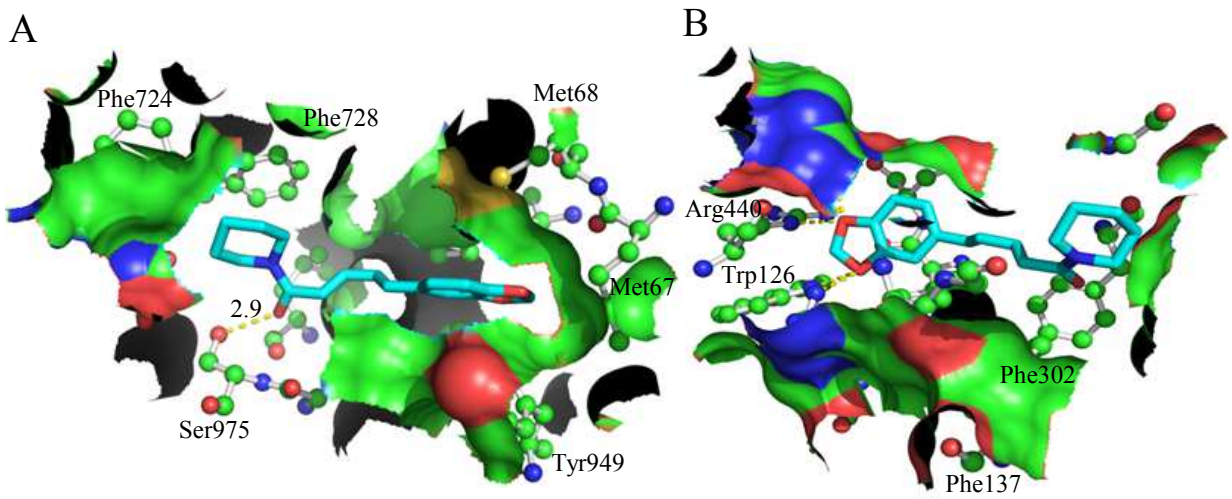


Fig. 4. Piperine binding to the P-gp transporter (A) and P450 3A4 cytochrome (B) is shown within the protein binding sites represented by a molecular surface with the interacting amino acid residues. The piperine molecule is depicted in sticks; and the protein residues are displayed as ball-and-stick models, respectively. Hydrogen bonds are visualized as dashed lines measured in Å. All Hydrogen atoms are removed to enhance the overall clarity. The high-resolution graphics were prepared using the AutoDock and PyMol software [144, 153].

In fact, this compound has already proven to be active against different bacteria [154], so its derivative forms, including piperonal, piperonylic and piperic acids have shown the similar effects [155, 156]. Some inhibitory effects of piperine were confirmed in the experiment for breast cancer in combination with epigallocatechin gallate, using mouse macrophages [157]. Furthermore, a large library of piperine analogs, using the Autodock and Authodock Vina software, was screened for the possible hit and lead compounds to bind to survinin as a member of the inhibitor of apoptosis family [158]. Other results from the molecular dynamics simulations using the MM-PB/GBSA (molecular mechanics Poisson-Boltzmann and generalized Born surface area) approach together with the alanine scanning defined the important role of hydrophobic interactions as a driving force in the piperine-protein binding [159].

Moreover, the piperine cytotoxic potential and its anti-HIV activity were determined in the combination with the QSAR approach [160]. Furthermore, using the computational approaches to predict the peperine toxicity *in vivo* might be also beneficial for the animal welfare to reduce the unnecessary usage of laboratory animals [161]. Some other studies used QSAR to analyze the piperine analogs to inhibit the NorA efflux pump in Staphylococcus to predict the protein-

ligand binding mechanism and to measure quantitatively the ligand binding affinity to NorA [145].

In conclusion, phytochemical and pharmacological attributes of piperine as an active pharmaceutical ingredient indicated its value for pharmaceutical chemistry and biomedicine. Different synthetic strategies, extraction, and detection techniques emphasized the important role of piperine for the development of novel natural remedies and future perspectives towards its efficient formulation with hydrophilic excipients. In particular, some of these novel approaches for optimizing delivery of piperine based on its complexation with CD and interaction with the P450 3A4 cytochrome and P-gp transporter were discussed. In addition, the combination of theoretical and experimental techniques might pave the road to more effective biomedical and pharmacological applications of piperine and its novel analogs in modern biomedical practice.

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Highlights:

1) Plant extracts contain antimicrobial properties to treat different pathogens

2) Phytochemicals are safe ingredients to develop novel plant-based pharmaceuticals

3) Piperine has the potential as dietary supplement together with therapeutic approaches

4) Combination of theoretical and experimental methods improves the piperine effectiveness in biomedicine