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Efficient Extraction and Structural Analysis of Biosourced Piperine as Natural Adjuvant †

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Abstract: In the framework of valorization of introduced and cultivated spices on Algerian soil, Piperine main constituent of black pepper is chemically and biologically studied, as it improves bioavailability of several synthetic and natural drugs such as Resveratrol and Curcumin thanks to its diffusion mechanism and a high permeability coefficient. In fact it was reported that Curcumin-Piperine nanoparticals were used to increase Curcumin bioavailability in cancers treatment. In the present work an efficient Soxhlet extraction of Piperine with several solvents screening namely: ethanol, chloroform, dichloromethane, acetate ethyl, acetone; and time depending is reported in order to optimize extraction conditions and maximize extraction yields, besides a purification and structural characterization of obtained biocompounds was conducted using several analytical and spectroscopic methods as: MP, TLC, UV, FT-IR. Optimized Soxhlet extraction exhibits ethanol in 2h as the best solvent and time extraction conditions. On the other hand, LC chromatography isolation in addition to spectroscopic analysis leads to identify target pure Piperine. The scope of this study is to use the obtained biobased Piperine in further applications like hemi synthesis or formulation by simply encapsulated and used as nutraceutical adjuvant to optimize efficiency of other biomoleculs

Keywords: bioenhancer; piperine; solvent screening; soxhlet extraction; purification; structure analysis

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1. Introduction

Piperine is found in black pepper (piper nigrum), white pepper, and long pepper (piper longum) belonging to the family Piperaceae. It has a distinctive pungent flavor due to the presence of an alkaloid Piperine, along with volatile oils, and essential oils. The content of Piperine varies from plant to plant belonging to the Piperaceae family and varies from 2% to 7.4% in vines of black and white pepper (piper nigrum) [1]. The amount of Piperine content can be influenced by modifications in conditions of cultivation such as climate or drying conditions and the place of origin [2].

It is commonly used as food condiment used as medicine, preservative, perfume and also in human diet. Piperine is weakly basic in nature, which on hydrolysis (acidic/basic), can be converted to piperic acid and piperidine [3]. A conjugated aliphatic chain acts as a bridging connective structure between piperidine and 5-(3,4-methylenedioxyphenyl) moiety. This makes Piperine a unique and excellent molecule to offer optimum attributes for the tendency of the molecule to bind successfully to enzymes (Figure 1). Black pepper contains four isomeric forms of Piperine, increases with an increase in light intensity and time exposure. Light-induced isomerization can be seen in Piperine, transformation can also be seen on storage that slowly and spontaneously leads to the loss of pungency [4,5].

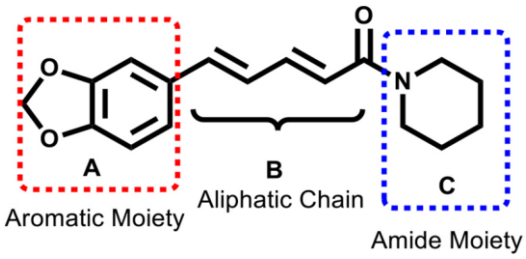

Figure 1. Piperine structure.

Piperine is used in traditional therapies [6] as well as in modern medicine; it is used in painmanagement, chills, rheumatism arthritis, influenza, and fever [7,8]. the enhancement of blood circulation, salivation, and stimulation of appetite [9,10]. It also acts on many enzyme systems (including p-glycoproteins) [11,12]. Becides, it has shown various biological activities such as anti-infective, antimicrobial, insecticidal, anti-inflammatory, antiamoebic, antiulcer, and antidepressant [13–22]. Moreover, Piperine can be highlighted due to its anti-cancer and anti-MDR activities. It is able to modulate the MDR phenotype in some experimental models, such as breast, lung, colon and lymphoma cancer. [23,24]. its action has been demonstrated in preclinical studies, by enhancing the cytotoxic effect of the chemotherapeutic drugs doxorubicin and mitoxantrone. [25–28]. In addition, it is suggested that Piperine can be used as an alternative medicine in the treatment of diseases related to obesity, process of digestion, antioxidant properties, and the role in the management of various disorders [29–33].

Piperine also increases the absorption and bioavailability of various drugmolecules when combined with a particular therapeutic agent such as rifampicin, simvastatin, ibuprofen, and omeprazole. without exerting any of its biological activity at the used dose. [34–36]. Which make it an excellent Bioenhancer agent capable of increasing the absorption from the gastrointestinal tract or inhibiting enzymes involved in the biotransformation of the drug by synergy, preventing the drug transformation to metabolites and by decreasing the rate of elimination [37].

It was listed by FDA as a safe herb for its use as a spice, in fact it can be given at a dose of 15–20 mg/person/day in divided dose, which seems to be extremely less than the LD50 of the human dose. Thus, reducing side effects and toxicity of drugs due to a lower dose and increased availability at the active site [38].

In the current work, bioenhancing properties, extraction, isolation, structure analysis and discussion on formulations target advances, of Piperine are reported. The future perspective are hemisynthesis, mechanism by which Piperine enhances the bioavailability and the preparation of advance pharmaceutical formulations.

2. Experiments

2.1. Plant Materials

Black paper seeds were commercially purchased from a local traditional shop, cleaned, dried and sprayed into a powder with an electrical pulvirisator until content was about 15g dry weight. All other solvents and reagents used in this work were of analytical grade and purchased from Sigma-Aldrich Company Ltd. (Germany).

2.2. Extraction

Solvent extraction was carried out using a Soxhlet.

Briefly, it consisted into the extraction of the hole crud of seeds (15 g) with 250 mL of several solvents: ethanol, chloroform, dichloromethane, acetate ethyl, acetone; for 2 h, and then filtered with a Büchner leading to crud extract, the filtrates were evaporated to concentrates using rotary evaporation at 40 °C and stored in an amber coated bottle at 4 °C. The extraction yield was calculated as follows:

Yields are calculated according to the following formula:

$$\text{Yield \%} = (\text{Crude extract mass/powder mass}) * 100$$

2.3. Isolation, Purification and Recrystallization

Obtained dry extract is transferred in tube assay and treated by 12 mL of ethanol, 12 mL of KOH (10%) and drip of cold water, then placed in ice bath overnight. Afterward, the mixture is filtered and washed with 45 mL of iced water and 45 mL of cold petroleum ether then recrystallized twice in a minimum of hot acetone.

2.4. Alkaloids Test (Dragindorff)

2 mL of ethanol extract is treated by diluted HCl, then a drop of Dragindorff reactif is added.

3.2. Thin Layer Chromatography

A rapid identification of bioactive compounds was done using thin layer chromatography (TLC). A chromatographic tank was filled with several solvents' systems to screen which is the best, and kept covered for 10 min to get saturated with solvent's vapors. Silica Gel 60 F254 precoated aluminum TLC plate 10 × 2 cm (Merck, Germany) was used as a stationary phase. The crude concentrated extract was loaded on the TLC plate and put in the tank containing solvent. Physic revelation of spots was done using U.V lamp at 254 and 365 nm, R*f* values were then calculated according to Touchstone [39].

3.4. Identification and Characterization

- Fourier transform infrared spectroscopy (FT-IR) analysis

For Infrared (IR) spectra of isolated compounds, 1 mg of powder was mixed with KBr to form a plate. Absorption peaks of functional groups were recorded using Fourier transform spectrometry instrument SHIMADZU HYPER, FTIR-8201 PC in the range of 4000–400 cm^{-1}.

- UV-VIS spectroscopy

UV-Visible absorbance was performed in scan mode with a SHIMADZU UV 16A spectrophotometer.

- Melting point

The melting points of isolated compounds were recoded on a digital apparatus type STUART.

3.5. Statistical Analysis

Each experiment was performed in five replicates and the data was subjected to calculations of mean ± S.E. The mean values were used for drawing the graphs.

4. Results and Discussion

4.1. Extraction Solvents Screening and Extraction Yields

According to obtained results we can observe that ethanol is the best extraction solvent with 12.34% of yield recorded in 2 h of Soxhlet extraction.

4.2. Isolation of Bioactive Compounds from Crud Extract

Black paper ethanol percolate extract (12.34%), was subjected to a purification and recrystallization of main bioactive compound which was obtained as light yellow crystals.

- The Dragindorff test to identify presence of alkaloids gave an orange precipitate which indicates positive results.

- TLC chromatography with a hexane/acetate diethyl (2/2.5) eluent system exhibit a characteristic spot Rf, which can be attributed to main bioactive compound of black peper Piperine [40] and is consequently chosen for further characterization and validation using, FT-IR, melting point and UV-VIS analysis.

4.3. Identification and Characterization of Main Bioactive Compound through FT-IR, Melting Point and UV-VIS

- The FT-IR spectra of isolated compound revealed characteristic absorption peaks in KBr pallets at 1582 and1584.58 cm-1 (C═C aromatics), 1254 and 1184 (C-O-C), 1700 cm-1 (-CO-N), 1634 and 1583 (C=C aliphatic). Close to those reported in literature [41].
- Melting point

Piperine Melting point was observed at 128°C, in nice agreement with literature (128-130°C) [41], which indicates the purity of obtained compound.

- UV-VIS

UV-VIS spectra of isolated Piperine was performed in ethanol/HCl (1V/1V) solution and recorded in the region of 800–200 nm at room temperature, it reveled an absorption band around 344nm, corresponding to $\pi \rightarrow \pi^*$ transition, which is in nice agreement with literature [41].

Therefore, the isolated compound was identified as (2E, 4E) -5- (benzo [d] [1,3] dioxol-6-yl) -1- (pipéridin-1-yl) penta-2,4-diène- 1-one).

5. Perspectives of Formulation

The Piperine use as nutraceutical is limited by low aqueous solubility, this lack of solubility can be improved by the use of solid lipid nanoparticles because of its low toxicity, biodegradable and biocompatible properties, in addition solid lipid nanoparticles possess unique properties like small size, large surface area and high drug loading ability. Thus, the solubility of Piperine can be enhanced by formulating them as solid lipid nanoparticles by using different preparation techniques so it can be effectively used for diverse applications in dermacosmetics, nutraceuticals, bioenhanceur of pharmaceutics...

6. Conclusions

In the present work, an efficient Soxhlet extraction, isolation and identification of Piperine the main bioactive compound of black paper through conventional routine methods were reported, in order to validate the ease access to this prized bioactive compound even at industrial scale, for pharmaceutical and food purposes using specific formulation to maximize its therapeutic effect as bioenhanceur.

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