WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 11, 764-776. Review Article ISSN 2277–7105

EXPLORING EXTRACTION, ISOLATION AND SCREENING METHOD FOR BIOACTIVE COMPOUND FROM BLACK PEPPER (PIPER NIGRUM L.)

Anupam Yadav*, Parul Shukla, Prachi Mishra, Sneha Pandey, Mehemmud Allam Kuresi, Shubhankit Soni and Himanshu Singh

Babu Sunder Singh College of Pharmacy, NH24, Raebareli Road, Nigohan, Lucknow (226302).

Article Received on
16 April 2024,

Revised on 06 May 2024,
Accepted on 26 May 2024

DOI: 10.20959/wjpr202411-32668

***Corresponding Author**
Anupam Yadav
Babu Sunder Singh College of Pharmacy, NH24, Raebareli Road, Nigohan, Lucknow (226302).

ABSTRACT

To delve to all of the methods used to extract, separate, and filter black pepper (Piper nigrum L.) in order to identify its bioactive components. Black pepper is a popular spice because of its distinct taste and potential health benefits. Black pepper's natural participants, particularly terpenes, flavonoids, and alkaloids, have been demonstrated to possess anti-inflammatory, antibacterial, and antimicrobial qualities. The study covers a variety of extraction procedures, including basic methods such as Soxhlet extraction and maceration. Black pepper may be extracted more successfully with the use of these methods since they maximize yield while sustaining the chemical integrity of the bioactive components.

To separate and refine certain bioactive components from complex pepper extracts, techniques such as thin-layer chromatography and column chromatography are applied. The study emphasizes the use of testing methods to evaluate the biological activity of the surrounded chemicals. Among the methods employed are antioxidant tests, antimicrobial testing, anti-inflammatory assays, and anticancer monitoring. The screening process helps identify the specific bioactive compounds liable for the biological activities that are observed.

KEYWORDS: Black Pepper, Piperine, Extraction, Maceration, TLC, Microscopy, UV.

INTRODUCTION

Piper nigrum L., often referred to as black pepper, is a popular spice that is frequently used in cuisine. Due to the presence of bioactive components, black pepper has been shown to have potential medical advantages beyond its culinary value. These bioactive substances, which include alkaloids, flavonoids, and terpenes, have been demonstrated to have a variety of therapeutic benefits, including antibacterial, anti-inflammatory, antioxidant, and anticancer effects. In order to achieve the promise of black pepper, effective methods for the extraction, isolation, and screening of its medicinal properties must be developed. Extraction techniques are essential for obtaining concentrated portions of black pepper, whilst isolation methods help separate and purify particular ingredients from multifaceted mixtures.

Once being isolated, these compounds go through screening processes to determine their biological activity and possible therapeutic potential. To extract its bioactive ingredients, black pepper is processed via maceration, Soxhlet extraction, supercritical fluid extraction, and microwave-assisted extraction. Similarly, it looks at isolation techniques including preparative chromatography, solid-phase extraction, thin-layer chromatography, and column chromatography that are used to purify certain molecules. The article also looks at a number of screening techniques for determining out these molecules' bioactivities.

Such as antioxidant assays, antimicrobial testing, anti-inflammatory assays, and anticancer screens. by delving into the extraction, isolation, and screening methods for bioactive compounds from black pepper, this review paper aims to contribute to the understanding of the potential health benefits associated with this spice. It also highlights the importance of these methods in the development of new pharmaceuticals, nutraceuticals, and functional food products derived from black pepper.

Figure 1: Black pepper.

Methods of Extraction and Isolation of piperine

Soxhlet deletion

The substance- 95% ethanol, black pepper, KOH 10% alcoholic, Soxhlet apparatus.

The method

Grind 10g of black pepper into a fine powder.

⬇

Placed into a round-bottom flask holding 200 ml of 95% ethanol.

⬇

Place the RBF on a heating mental and set the Soxhlet apparatus.

⬇

Continue the extraction for three hours.

⬇

Use filter paper to filter the ethanolic extract. Concentrate the extract till 5 ml.

⬇

Add 10 ml of KOH solution (10% alcoholic)

⬇

Hold onto the key combination for an hour.

⬇

Pour the alcohol solution out of the residue that has precipitated

⬇

Wait 24 to 48 hours for the piperine crystals to form

⬇

After applying 95% alcohol to clean it, let it air dry and weigh them

Yield:- 0.7 gram

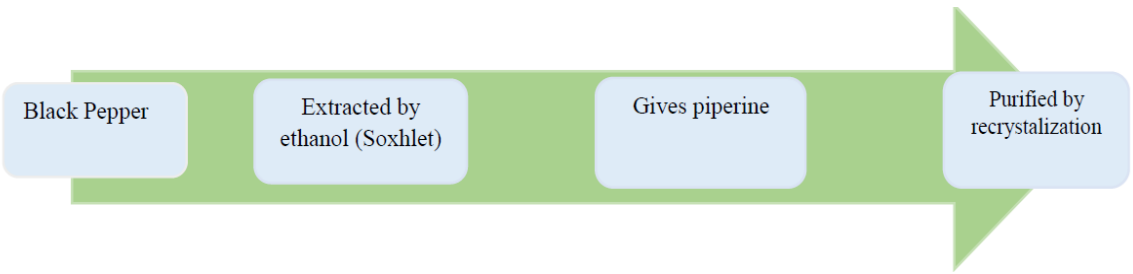

Figure 2: Soxhlet apparatus.

Maceration

The substance- Black pepper, Sodium bicarbonate, Glacial acetic acid, Chloroform, Di-ethyle either.

The method

10g of black pepper peppercorns were crushed.

⬇

Add 50 cc of glacial acetic acid and mix.

⬇

Store the two together for a full day.

⬇

After filter out content, dilute the acetic acid extract with 50 milliliters of chloroform.

⬇

Added three times to a separating funnel wash with 50 cc of chloroform

⬇

Use distilled water to rinse the whole thing. Four or five times

⬇

Used ten percent sodium bicarbonate to wash the chloroform extract.

⬇

Filtered the extract through anhydrous sodium sulphate, dehydrated using a rotatory

⬇

Evaporator, and the residue was reconstituted in 3 ml chloroform with 30 ml diethyle added.

⬇

The solution then remained at 4 °C for 8 hours,

⬇

Yielding creamy white crystals.[2]

Yield- 0.8gm

Figure 3: Separating funnel.

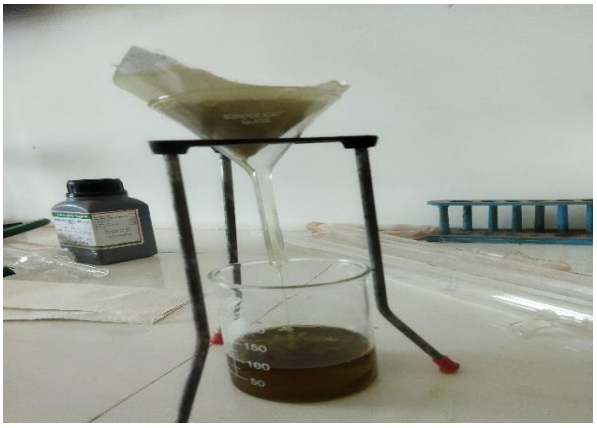

Figure 4: Filteration.

Piperine

Piperidine The dried fruit extract of pepper has been utilized by Danish scientist Hans Christian Orstedt in 1820 to isolate piperine (1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadieny] piperidine), an alkaloid molecule containing nitrogen. It was a yellow crystalline solid with a molecular weight of 285.33 g.mol−1, mp = 128–130 °C. Piperidine and the molecular building block of piperine molecules, the 5-(3, 4-methylenedioxyphenyl) moiety, are connected via conjugated aliphatic chains. Natural sources of piperine include white, green, and black peppers. Black pepper extracts also include other alkaloids, including piperanine, piperettine, piperyline A, piperyline B, and pipericine. The health benefits of piperine have drawn a lot of curiosity during the last 20 years.[9–11]

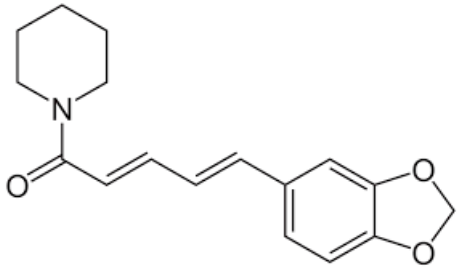

Figure 5: Structure of piperine.

Method of screening piperine

1. Thin layer chromatography

Solvent selection

Numerous factors, including as the properties of the material and the makeup of the stationary phase, influence the choice of solvent.

For instance, acetone toluene (8:2) is used as the solvent in the TLC extraction of piperine. A slurry is created by mixing the chosen adsorbent with water or another solvent. One example is a solution of silica gel G and water.

A glass slide of 20 cm by 20 cm is utilized, and the TLC plate is chosen after obtaining the necessary and meticulous measurements. Selecting a top-notch plate that can withstand high temperatures requires action.

The slurry must then be poured onto the TLC plate. The plate under contact. To activate the plate, the plate must be exposed to air.

Sample spotting: Make sure the spotting region is not submerged in the mobile phase when using a micropipette or capillary tube. Inserted the indicated slide into the solvent chamber with the 8:2 ratio of acetone to taluene. After enclosing the chamber, let the solvent to run for thirty minutes. Next, take the slide out of the chamber and put it in a UV cabinet that runs at 245 nm wavelength. A green dot will indicate the active component. After determining the solvent and sample's running distance, determine the RF value.

Figure 6: UV cabinet with slide.

RF value = Distance run by solute / Distance run by solvent

RF = 1.5/5

RF = 0.3

2. Microscopy

Sample preparation: Extract piperine from black pepper by using a solvent, such as methanol or ethanol. Recrystallize the extract to obtain pure piperine crystals.

Mounting: Place a little amount of piperine crystals on a microscope slide. Use a mounting medium, such as Permount, DPX, or Canada balsam, to secure the crystals in place.

Cover slipping: Cover the mounted sample with a coverslip to prevent dust and moisture from skewing the observation.

Microscopy: Use a scanning electron microscope (SEM) or polarizing light microscopy (PLM) to see the piperine crystals.

Plm: Measure the crystals' extinction angles and other optical characteristics by seeing them under polarized light.

Sem: Use a scanning electron microscope to examine the outer appearance and crystal structure of piperine.

Analysis and Observation: Transparent white solid crystals having a rigid structure having shine.

Numerous commonly utilized microscopy methods for piperine include: High-resolution images microscopy

Transmission electron microscopy (Tem)

Scanning electron microscopy (Sem)

Polarized light microscopy (PLM)

Elemental analysis employing energy

Dispersive X-ray spectroscopy (EDS)

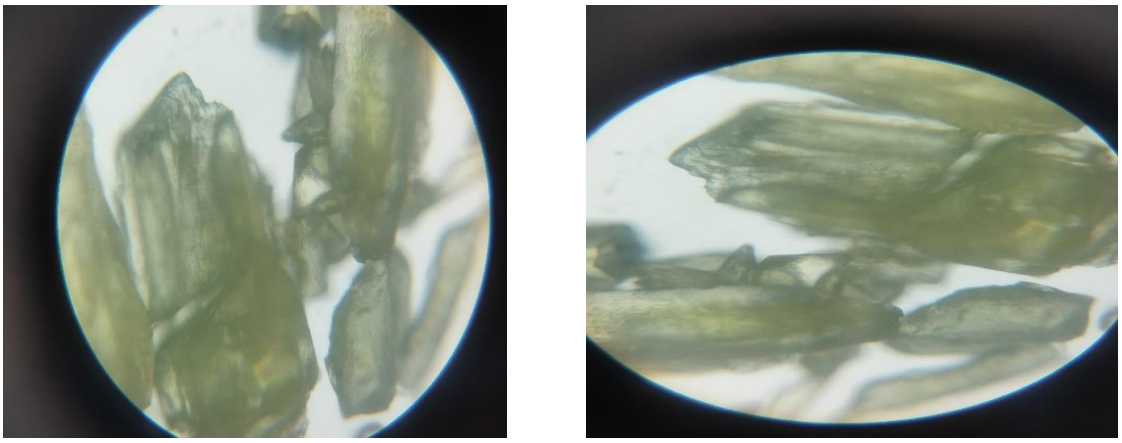

Figure 7: Microscopic view of piperine.

3. Ultra-voilet radiation

Sample preparation

Use a solvent such as ethanol to dissolve a known quantity of piperine. Create a solution that has a concentration of 1–10 mg/ml that is appropriate for UV analysis.

Setup of the instruments: Use a spectrophotometer with UV-Vis. Configure the device to measure transmittance or absorbance in the UV (200–400nm) range.

An example of an analysis: The piperine solution should be put in a quartz cuvette. In the spectrophotometer, install the cuvette. Note the sample's UV spectrum.

Data analysis

Identify absorbance maxima λ max and corresponding absorbance value

λmax 1: around 342-345 nm (In ethanol)

λmax 2: around 257-262 nm (in ethanol)

Morphology of piperine

Piperine is an alkaloid found in black pepper.

Chemical formula: **C17H19NO3**

Melting point: **285.34 g/mol**

A complex structure ring include a piperidine ring (Six membered ring containing a nitrogen atom) with a long hydrocarbon chain made up of 17 carbon atoms. This chain gives lipophilic properties.

Appearance: A yellowish crystalline solid. having distinct appearance with its bright yellow colour.

Figure 8: Piperine crystals.

Synthetic piperine analogs, SAR, and Chemical modification, Chemically speaking

Leishmania donovani pteridine reductase 1 (LdPTR1), an alkaloid, is made up of three subunits: a piperidine ring with α-β-unsaturated carbonyl moiety, 1A; a butadiene chain; a 1,3-benzodioxole group, also known as the piperonal nucleus; and all four of piperine's isomers, with isochavicine showing the most inhibitory action. It becomes revealed that TRPV1 and TRPA1 were activated by isochavicine, piperine, and isopiperine. Several studies have found a large number of piperine derivatives and analogues, as well as their biological characteristics and structure–activity relationship (SAR). The piperidine moiety can be replaced with N, N-dipropyl, p-methyl piperidine, N, N-diisopropyl, N, N-dibutyl, and other drugs that increase the therapeutic efficacy of piperine derivatives.[12–19]

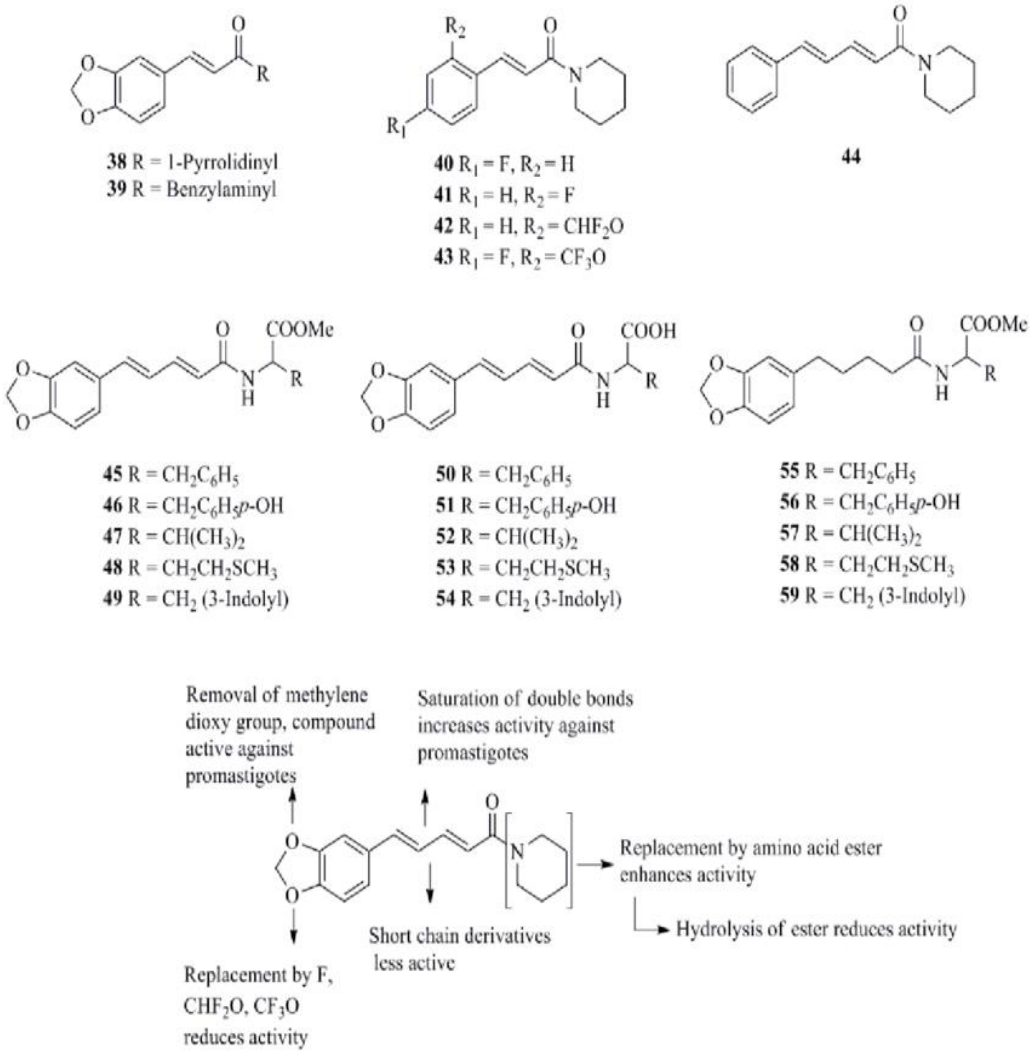

Figure 9: Structure activity relationship of piperine.

Drugs with piperine, Route and Detection methods

Drug	Piperine + Drug timing	Roa	Methods of detection
Propranolol	7 days, 40 mg + 20 mg	By mouth	Spectrofluorimetric method
Theophylline	7 days, 150 mg + 20 mg	By mouth	EMIT
Diclofenac	10 days, 100 mg + 20 mg	By mouth	Phoenix Win Nonlin 6.2 software, NCAM
Z- Chloride	10 days, 200 mg + 20 mg	By mouth	Phoenix®, Win Nonlin 6.4® software, NCAM
Emodin	1 day, 20 mg/kg + 20 mg/kg,	By mouth	LC–MS/MS
Linarinec	1 day, 50 mg/kg + 10 mg/kg	By mouth	DAS 2.1.1 Software, ANOVA, NCAM
Curcumin (Turmeric)	1 day in rats- 2g/kg + 20 mg/kg 1 day in humans- 500 mg + 50 mg	By mouth	PHARMKIT computer programme with SIMPLEX algorithm, MIM
Cannabidiol	10 days, 15 mg/kg + 10 mg/kg,	By mouth	Win Nonlin® (version 5.2, Pharsight, Mountain View,

			CA), NCAM
Fexofenadine	1 day, 5 mg/kg + 10 mg/kg 10 mg/kg + 10 mg/kg	By mouth + IV	Win Nonlin ® (version 5.2, Pharsight, Mountain View, CA), NCAM
Sodium valproate	1 day, 150 mg/kg + 15 mg/kg	By mouth	trapezoidal method NCAM
Oxoretinol	100 mg/kg + 10 mg/kg 1 day, 10 mg/kg + 1 mg/kg	By mouth/ IV	PK Solution 2.0 software (Summit Research Service) NCAM

Piperine application: Using piperine might make it simpler to get essential nutrients. It could also be beneficial for supporting weight and reducing anxiety, among other things.

1) **Helpful in the reduction of stress:** Since piperine increases the synthesis of catecholamines, including adrenalin, it could quickly help manage stress. Buffered vitamin C is commonly used in conjunction with piperine.

2) **Support with weight control:** Piperine probably possesses thermogenic properties that increase basal metabolic rate in the body. Each of these features may help you manage a healthy weight.

3) **Improving bioavailibility:** Essential components that piperine may make it simpler to absorb include beta-carotene, curcumin, selenium, and vitamin B6. It could also enhance the absorption of amino acids.

4) **More benefit:** Apart from all of these possible benefits, piperine can also help the digestive system and maintain healthy breathing patterns. Additionally, it may help treat joint problems and gastrointestinal ulcers.

CONCLUSION

Using a solvent, such as methanol or ethanol, is an accepted technique for extracting the active components from black pepper. The active compounds dissolve into the solvent through a process named maceration that happens when the solvent and crushed pepper are mixed together. This liquid is then filtered to remove any remaining solids. The extracted extract eventually undergoes a process called fractionation. This means sifting through the extract's many ingredients. Chromatography is often utilized in this role. It separates the mixture according to the various affinities of the chemicals for the stationary and mobile phases. Researchers may now access certain molecules or categories of compounds thanks to this. Once the chemicals have been separated, their biological activity may be evaluated. This

means evaluating the impact of the substances via a range of techniques and tests. In particular, DPPH or ABTS assays are methods that researchers might employ to measure antioxidant activity. The anti-inflammatory properties can also be evaluated using cell-based experiments or animal models. These methods for extraction, isolation, and screening help researchers identify and understand the active components in black pepper. It's a novel strategy to see what possible health advantages this spice might have.

REFERENCES

1. Benedikt A. Weggler. Frank L. Dorman, in Separation Science and Technology, 2020.
2. Amit Kumar Tripathi, Anup Kumar Ray & Sunil Kumar Mishra *Beni-Suef University Journal of Basic and Applied Sciences.*
3. Gorgani L, Mohammadi M, Najafpour GD, Nikzad M Piperine—the bioactive compound of black pepper: from isolation to medicinal formulations. Compr Rev Food Sci Food Saf, 2017; 16: 124–140.
4. Aziz NS, Sofian-Seng N, Mohd Razali NS, Lim SJ, Mustapha WA A review on conventional and biotechnological approaches in white pepper production. J Sci Food Agric, 2019; 99: 2665–2676.
5. Buckle KA, Rathnawathie M, Brophy JJ Compositional differences of black, green and white pepper (*Piper nigrum* L.) oil from three cultivars. Int J Food Sci Technol, 2007; 20: 599–613.
6. Takooree H, Aumeeruddy MZ, Rengasamy KRR, Venugopala KN, Jeewon R, Zengin G, Mahomoodally MF A systematic review on black pepper (*Piper nigrum* L.): from folk uses to pharmacological applications. Crit Rev Food Sci Nutr, 2019; 59: S210–S243.
7. Tiwari A, Mahadik KR, Gabhe SY Piperine: a comprehensive review of methods of isolation, purification, and biological properties. Med Drug Discovery, 2020; 7: 100027.
8. Derosa G, Maffioli P, Sahebkar A Piperine and its role in chronic diseases, 2016; 173–184.
9. Meghwal M, Goswami TK Piper nigrum and piperine: an update. Phyther Res, 2013; 27: 1121–1130.
10. Singletary K Black pepper. Nutr Today, 2010; 45: 43–47.
11. Schöffmann A, Wimmer L, Goldmann D, Khom S, Hintersteiner J, Baburin I, Schwarz T, Hintersteininger M, Pakfeifer P, Oufir M, Hamburger M, Erker T, Ecker GF, Mihovilovic MD, Hering S Efficient modulation of γ-aminobutyric acid type a receptors by piperine derivatives. J Med Chem, 2014; 57: 5602–5619.

12. Koul S, Koul JL, Taneja SC, Dhar KL, Jamwal DS, Singh K, Reen RK, Singh J Structure-activity relationship of piperine and its synthetic analogues for their inhibitory potentials of rat hepatic microsomal constitutive and inducible cytochrome P450 activities. Bioorganic Med Chem, 2000; 8: 251–268.
13. Sattarinezhad E, Bordbar AK, Fani N Piperine derivatives as potential inhibitors of Survivin: an in silico molecular docking. Comput Biol Med, 2015; 63: 219–227.
14. Correa EA, Högestätt ED, Sterner O, Echeverri F, Zygmunt PM In vitro TRPV1 activity of piperine derived amides. Bioorganic Med Chem, 2010; 18: 3299–3306.
15. Dong Y, Yin Y, Vu S, Yang F, Yarov-Yarovoy V, Tian Y, Zheng J A distinct structural mechanism underlies TRPV1 activation by piperine. Biochem Biophys Res Commun, 2019; 516: 365–372.
16. Eigenmann DE, Dürig C, Jähne EA, Smieško M, Culot M, Gosselet F, Cecchelli R, Cederberg Helms HC, Brodin B, Wimmer L, Mihovilovic MD, Hamburger M, Oufir M In vitro blood–brain barrier permeability predictions for GABAA receptor modulating piperine analogs. Eur J Pharm Biopharm, 2016; 103: 118–126.
17. Wimmer L, Schönbauer D, Pakfeifer P, Schöffmann A, Khom S, Hering S, Mihovilovic MD Developing piperine towards TRPV1 and GABAA receptor ligands - synthesis of piperine analogs via Heck-coupling of conjugated dienes. Org Biomol Chem, 2015; 13: 990–994.
18. Zabela V, Hettich T, Schlotterbeck G, Wimmer L, Mihovilovic MD, Guillet F, Bouaita B, Shevchenko B, Hamburger M, Oufir M GABAA receptor activity modulating piperine analogs: In vitro metabolic stability, metabolite identification, CYP450 reaction phenotyping, and protein binding. J Chromatogr B Anal Technol Biomed Life Sci, 2018; 1072: 379–389.