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Analysis of the Molecular Docking of Piperine with Cyclooxygenase-2, Followed by an *In-Vivo* Study of Its Anti-Inflammatory and Analgesic Properties

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ABSTRACT:

Piperine, the primary alkaloid in black pepper (*Piper nigrum*), is known for its promising biological activities. However, traditional extraction methods often face challenges such as low yield, high costs, and the risk of photodegradation. This study aimed to develop a simple, rapid, and efficient method for isolating piperine. Ethanol was used as the solvent for extraction, followed by isolation and purification through the Soxhlet method, which provided high-purity crystalline piperine, confirmed by physicochemical and spectroscopic analyses. In silico docking studies revealed that piperine forms a more stable complex with cyclooxygenase-2 (COX-2) than the native ligand (arachidonic acid), as evidenced by lower ΔGbind values. In vivo experiments demonstrated that piperine, administered at 50 mg/kg body weight, exhibited significant anti-inflammatory and analgesic effects within 60 to 180 minutes, with inhibition percentages comparable to aspirin at 150 mg/kg. This study successfully developed an efficient extraction method for piperine. It confirmed its significant biological activities, including enhanced COX-2 binding and practical anti-inflammatory and analgesic effects, highlighting its potential as a natural alternative to conventional treatments.

Keywords: Black pepper; Piperine; Extraction; In silico.

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1. Introduction

Natural products, particularly those derived from plants, have been a key source of therapeutic agents since antiquity. Today, approximately 25–30% of approved drugs originate from natural compounds, highlighting their continued importance in drug discovery [1]. Among these, spices—classified as medicinal plants—are rich in bioactive compounds with diverse pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory effects [2].

Black pepper (Piper nigrum), one of the most widely used spices, contains piperine, an alkaloid with significant medicinal potential. Traditionally, black pepper has been employed in conventional medicine for its analgesic and anti-inflammatory properties [2].

In vitro studies have shown that piperine inhibits key inflammatory enzymes such as 5-lipoxygenase and cyclooxygenase-1, suggesting its potential role in modulating inflammation [3, 4]. Additionally, piperine exhibits cytoprotective, antioxidant, and neuroprotective activities [5]. Despite these promising pharmacological effects, data on piperine's analgesic, anti-inflammatory, and antipyretic properties remain limited [6, 7]. To address this gap, we aimed to isolate piperine from Piper nigrum and assess its potential as an anti-inflammatory and analgesic agent through molecular docking, molecular dynamics simulations with

cyclooxygenase-2, and in vivo evaluation in an animal model.

2. Materials & Methods

2.1. Isolation of Piperine

The black pepper fruits were purchased from a spice store in Wad El-Had, Daksi, Wilaya de Constantine, Algeria. (Country of harvest: India, production date: 2020, expiration date: 2024). Chemical reagents: Ethanol, Potassium hydroxide, Chloroform, Sodium bicarbonate. All chemicals and reagents used in this study were at least analytical grade.

2.2. Identification of Piperine

2.2.1. UV-Visible Spectroscopy

A piperine solution in methanol was analyzed between 200 and 400 nm. λ max observed was 344 nm, matching the reference.

2.2.2. Infrared Spectroscopy (IR)

ATR-FTIR analysis confirmed the presence of characteristic functional groups.

2.2.3. NMR Spectroscopy

H1 and C13 NMR spectra in DMSO-d6 confirmed the structure of piperine.

2.3. In Silico Study (Molecular Docking)

Molecular docking was performed using AutoDock Tools and Accelrys Discovery Studio. The structure of COX-2 (PDB ID: 1CVU) and ligands were prepared. The docking parameters included the Lamarckian genetic algorithm. Validation was achieved by redocking arachidonic acid (RMSD=1.23 Å).

2.4. In Vivo Study

2.4.1. Plant material

Piperine was extracted from the black pepper fruits using the Soxhlet method.

2.4.2. Chemical reagents and solutions

Aspirin (Lot N0 248719 684, FLUKA Laboratory); A solution of 2 g/l NaCl; A solution of 0.9% NaCl; A solution of 1% Acetic acid (CH3COOH): prepared in distilled water; A 1% Toluene solution: prepared in distilled water; Tween 80; A Triton solution.

2.4.3. Anti-Inflammatory Activity

Plantar edema was induced by carrageenan injection in mice. Three groups received saline (control), aspirin (150 mg/kg), or piperine (50 mg/kg). Paw volume was measured over 3 hours.

2.4.4. Analgesic Activity

The nociception threshold was measured after intraperitoneal administration of saline (control), piperine (150 mg/kg), or tramadol (30 mg/kg) at 30, 60, and 120 minutes.

2.5. Ethical Statement

The use of animals in this study was justified by the lack of viable alternatives to answer the research question. The number of animals used was minimized while ensuring the statistical validity of the results. The experimental procedures have been designed to minimize animal pain, suffering, and stress. Where possible, alternative methods (in vitro models) have been used. The animals were housed in optimal conditions under the Ethics and Professional Conduct Committee guidelines of Oran University, 1 Ahmed Benbella. Invasive procedures were performed under anesthesia, and analgesics were administered to minimize pain. The results, including failures and adverse reactions, will be published transparently. The experimental protocol has been reviewed and approved by the Ethics and Professional Conduct Committee of Oran University 1 Ahmed Benbella (reference: TP.145.007).

3. Results & Discussion

3.1. Isolation of Piperine

Piperine was isolated from black pepper using the Soxhlet method, which yielded a piperine yield of 3.1%.

Figure 1. Isolated piperine crystals.

The product obtained by the Soxhlet method produced clear needle-shaped piperine crystals with greater purity (Figure 1).

3.2. Characterization of Piperine

The isolated compound exhibited the typical reactions of alkaloids, yielding a blood-red color with concentrated

H_2SO_4 and a reddish-brown precipitate with Dragendorff's reagent. These results are consistent with the known reactivity of piperine and confirm the presence of a nitrogen-containing heterocycle.

Solubility tests showed that piperine was readily soluble in ethanol, slightly soluble in chloroform, and insoluble in water, which agrees with its reported solubility profile. The melting point was determined to be 130°C, which closely matches the literature value, confirming the purity of the isolated crystals.

UV-Vis analysis revealed maximum absorption at 344 nm and a secondary peak at 310 nm, approximately to the reference values (345 nm and 310 nm). This substantial

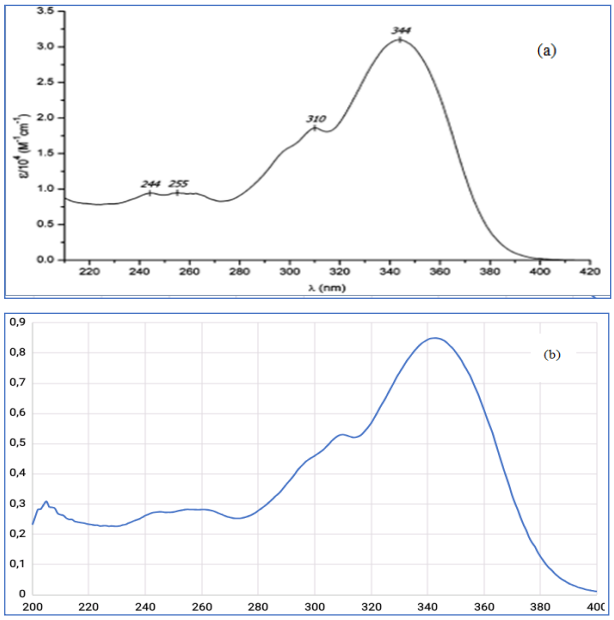

Figure 2. λ max of the reference piperine (a) (8) and of the piperine isolated (b) in methanol**.**

Table 1. IR peaks of isolated piperine.

Type of phenomenon	Standard values (8)	Values obtained
Aromatic C-H Stretch	3000	2943
C=C (diene) symmetric and asymmetrical	1635 1608	1663 1612
C=C Aromatic (Benzene)	1608 1580 1495	1612 1583 1509
–CO-N (Amide)	1635	1663
Flexion CH2	1450	1448
=C-O-C asymmetrical	1250 1190	1228 1194
Étirement symétrique =C-O-C Étirement C-O Flexion en plan du phényl C-H Flexion C-H du trans –CH=CH-	1030 930 1132 995	1031 929 1135 990

similarity supports the successful isolation of piperine without significant structural alteration (Figure 2).

IR spectroscopy further confirmed the identity of the compound (Figure 3). The characteristic bands corresponding to aromatic C=C stretching, C=O stretching of the conjugated ketone, and C-O-C vibrations of the benzodioxole group were observed and closely matched standard spectra (Table 1).

^{1}H NMR spectroscopy provided detailed structural confirmation. Signals corresponding to aromatic protons (7.9–7.2 ppm), vinyl protons (6.8–6.5 ppm), methylene protons of the O-CH_2-O group (~6.0 ppm), and piperidine ring protons (2.4–1.0 ppm) were all detected with the expected multiplicities. No unexpected signals were observed, indicating high purity and correct structural assignment (Figures 4-6).

The ^{13}C NMR spectrum showed signals between 20–50 ppm (sp^3 carbons of the piperidine ring), between 100–

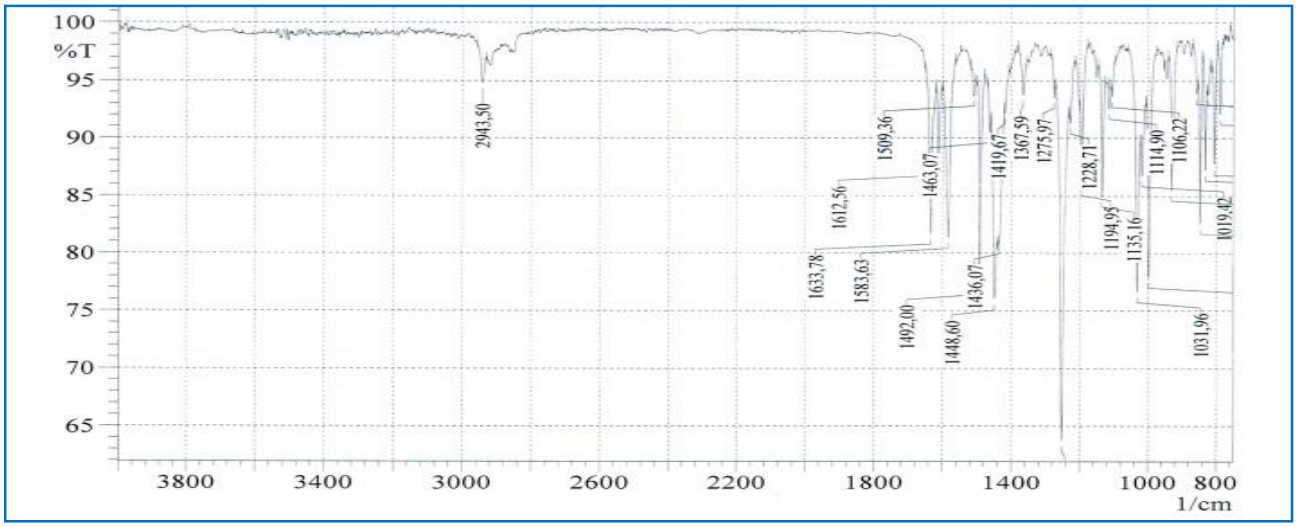

Figure 3. IR spectrum of isolated piperine.

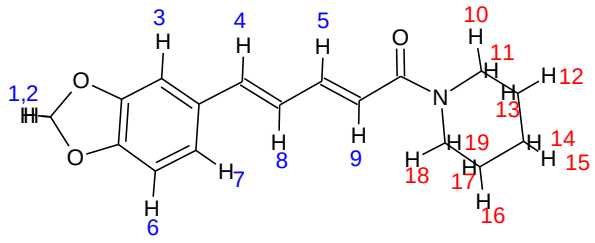

Figure 4. Types of protons in piperine.

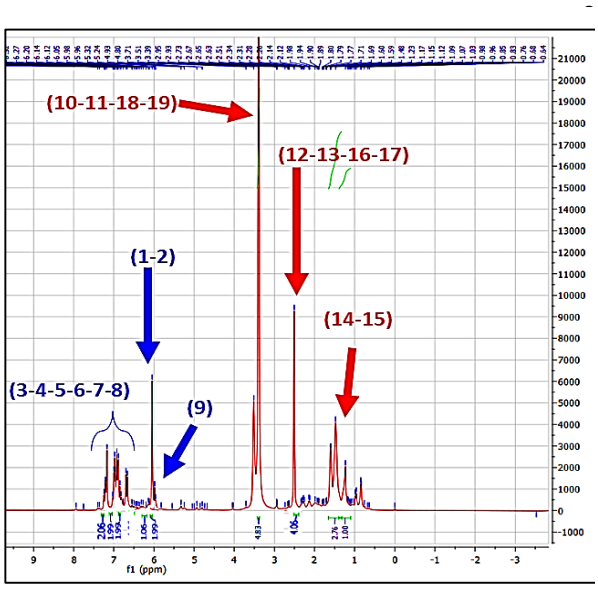

Figure 6. Experimental H1 NMR spectrum of the isolated piperine.

150 ppm (sp^2 carbons of the conjugated and aromatic systems), and a strong peak at ~165 ppm corresponding to the carbonyl group. The chemical shifts were highly consistent with theoretical values, supporting the assigned structure (Figures 7-9).

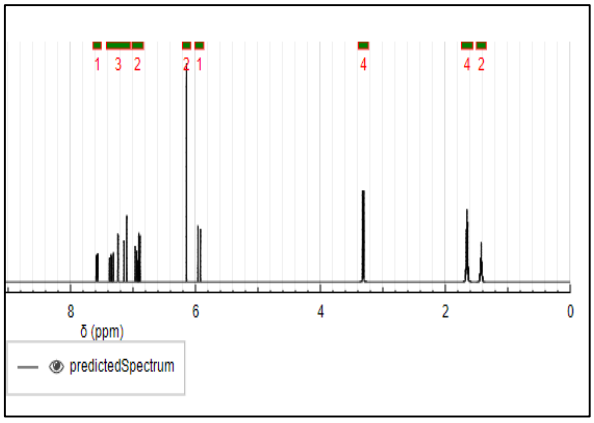

Figure 5. Theoretical H1 NMR spectrum [16].

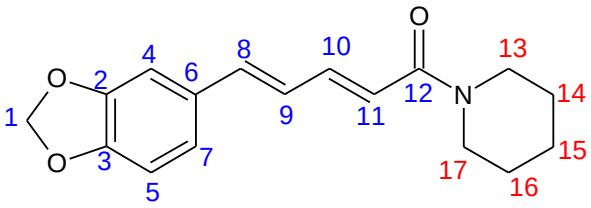

Figure 7. Types of carbons in piperine.

Overall, all analytical data converged toward the successful isolation and characterization of (2E,4E)-5-(1,3-benzodioxol-5-yl)-1-(piperidin-1-yl) penta-2,4-dien-1-one. The slight variations observed in the UV and NMR data (within 1–2 nm or ppm) are within acceptable experimental error and do not affect the structural assignment. These results confirm the efficiency of the extraction and purification protocols employed.

3.3. In Silico Study

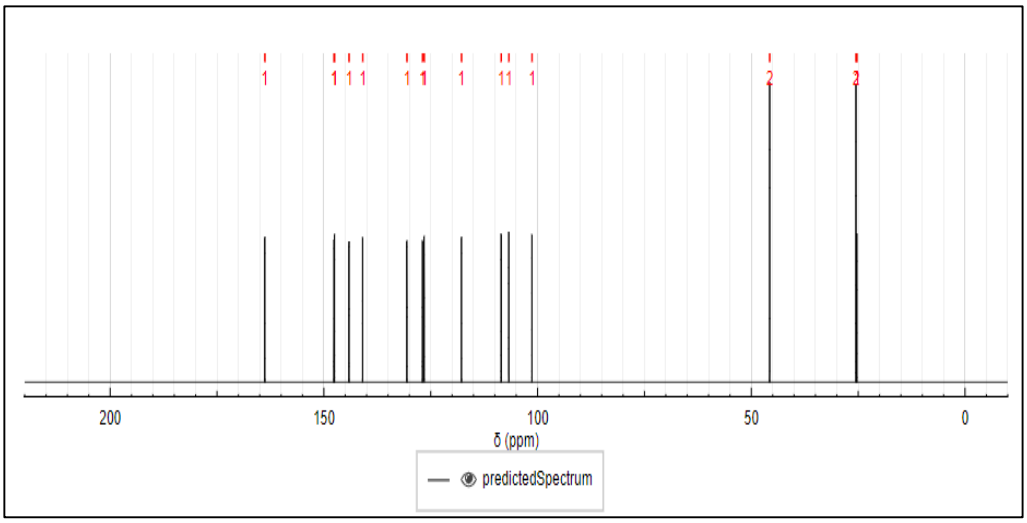

Figure 8. Theoretical C13 NMR spectrum.

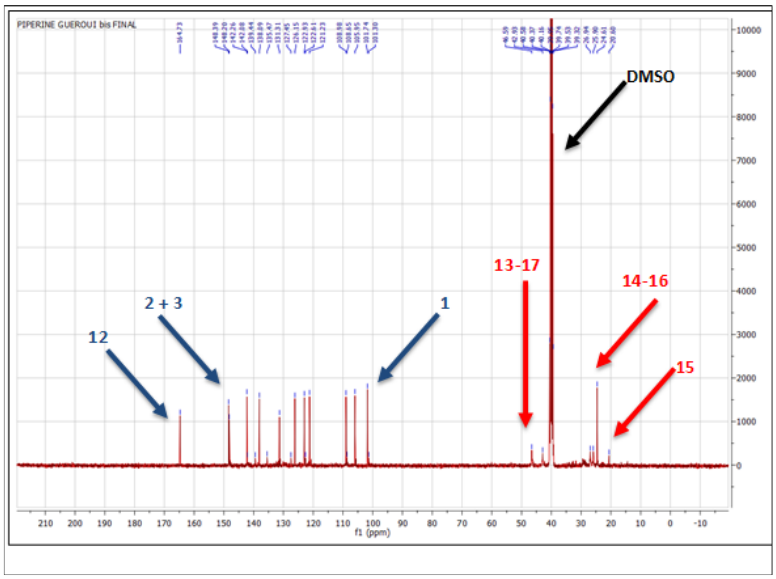

Figure 9. Experimental C13 NMR spectrum of the isolate

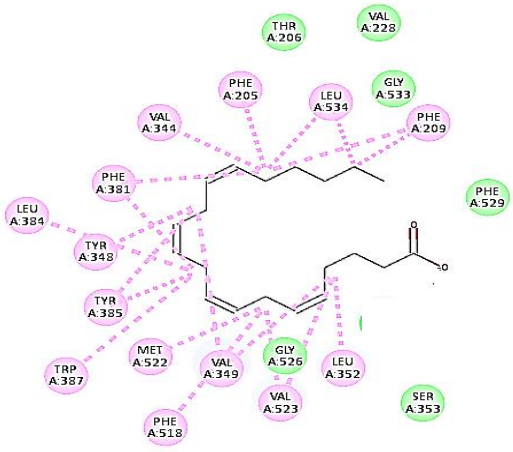

Figure 10. 2D pose of arachidonic acid on COX-2.

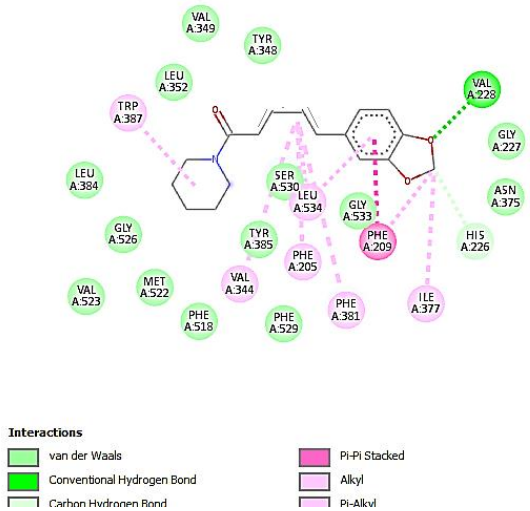

Figure 11. 2D pose of piperine on COX-2.

The docking protocol was successfully validated by redocking the native ligand, arachidonic acid, into the COX-2 active site, yielding a satisfactory RMSD value of 1.23 Å, confirming the docking method's reliability.

Table 2. Mean paw volume changes.

	Average volume of left posterior leg in ml			
Batch	Before injection	After injection of solutions		
		60 min	120 min	180 min
Witness (-)	0.1044±0.00648	0.140±0.00527	0.122±0.00976	0.1156±0.00988
Reference	0.1±0.00707	0.1044±0.00689*	0.1056±0.00503*	0.1089±0.1006
Piperine	0.0889±0.00889	0.0967±0.0085*	0.0933±0.0085*	0.0956±0.0093

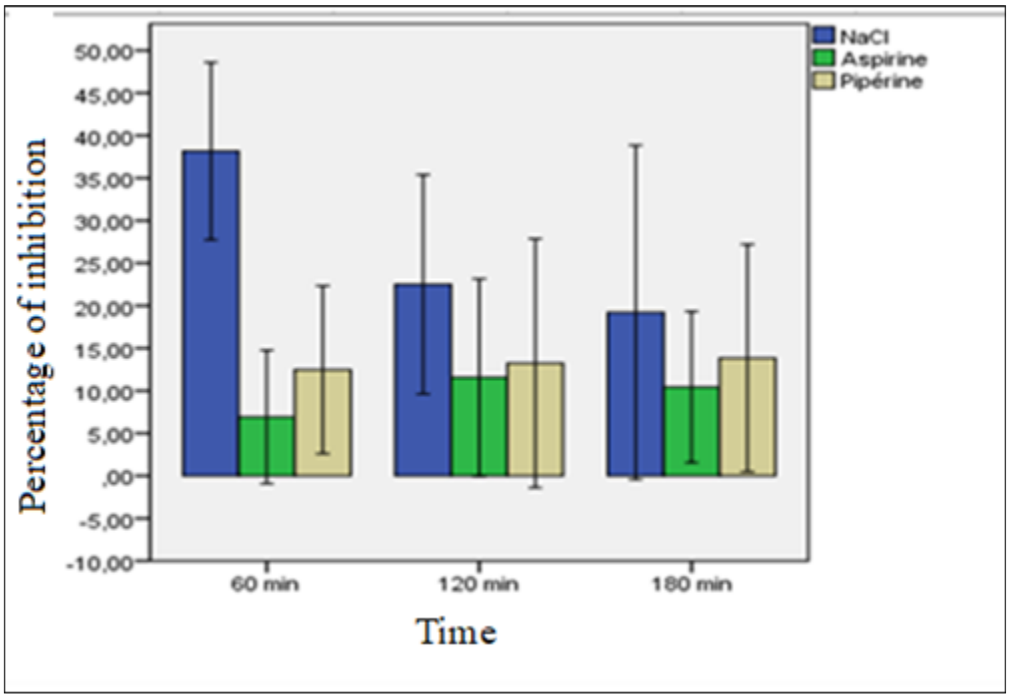

Figure 12. Edema evolution over time.

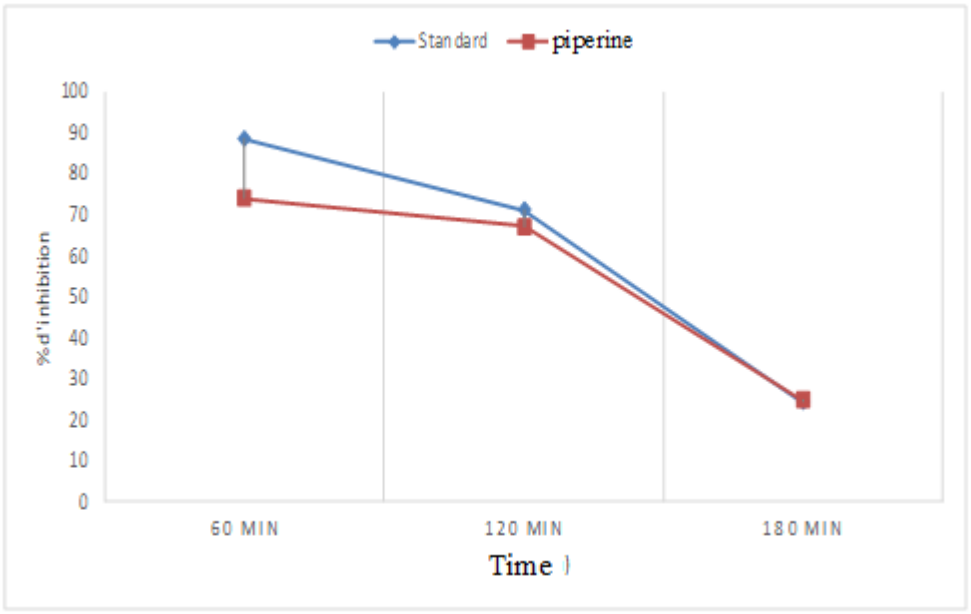

Figure 13. Edema inhibition percentages.

Piperine demonstrated a stronger binding affinity towards COX-2 compared to the native ligand. The binding energy improved from –8.68 kcal/mol for arachidonic acid to –10.23 kcal/mol for piperine, suggesting enhanced inhibitory potential.

Visual inspection of the docking poses revealed that piperine forms a key hydrogen bond with VAL228 at a distance of 2.40 Å. Additionally, several hydrophobic interactions involving residues ILE377, PHE381, PHE205, LEU534, VAL344, TRP387, PHE209, and GLY227 were observed, contributing to the stabilization of the Piperine-COX-2 (1CVU) complex. These interactions closely resemble those observed with arachidonic acid but with improved binding characteristics.

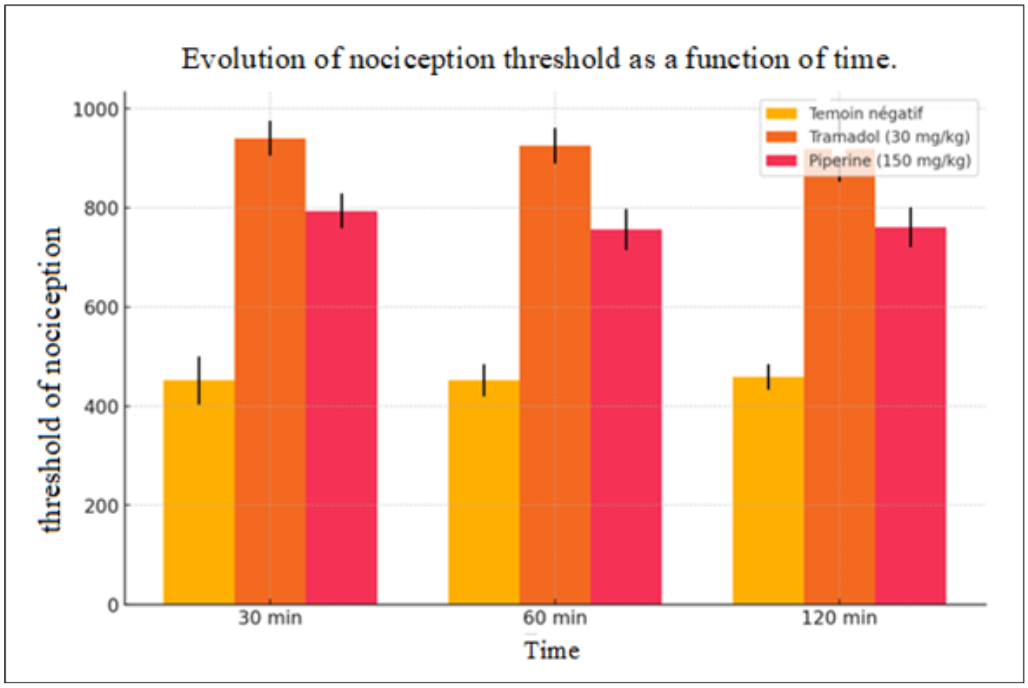

Figure 14. Nociceptive threshold evolution.

Furthermore, piperine exhibited a lower inhibition constant (Ki = 31.97 nM) than arachidonic acid (Ki = 233.06 nM), reinforcing the hypothesis of a higher inhibitory potency toward COX-2 (Figure 9-10).

3.4. Anti-Inflammatory Activity In Vivo

Edema progression was evaluated by measuring the increase in the volume of the left posterior paw over time (Table 2). A significant increase in paw volume was observed in the negative control group, whereas piperine and aspirin treatments significantly reduced the edema, as shown in Figure 12.

The anti-inflammatory activity was quantified by calculating the percentage of edema inhibition (Figure 8). The results, expressed as mean ± standard deviation (n=9 per group), demonstrated that piperine significantly inhibited carrageenan-induced inflammation, comparable to the effect observed with aspirin. Statistical analysis indicated significant differences compared to the control group ($p < 0.05$, Figure 13).

Edema induction by carrageenan remains a well-established model for studying acute skin inflammation and identifying anti-inflammatory agents. In our experimental conditions, piperine at a dose of 50 mg/kg inhibited edema formation from the first hour post-injection, with inhibition percentages approaching those achieved by aspirin at 150 mg/kg.

These findings corroborate previous studies by Jeffrey Raj et al. (2020) and Farhana Tasleem et al. (2014), which reported a significant anti-inflammatory effect of piperine using the carrageenan-induced paw edema model. Together, our data support the hypothesis that piperine exerts a marked anti-inflammatory effect, likely by interfering with the early mediators of inflammation.

3.5. Analgesic Activity In Vivo

The analgesic effect of piperine was assessed by measuring the animal's sensitivity to pressure-induced pain. A significant reduction in pain sensitivity was observed in the group treated with piperine, comparable to the effect of tramadol. These findings indicate that piperine exerts a protective peripheral impact, likely by interacting with specific pain-related receptors, as evidenced by the increased nociception threshold (Figure 14).

The observed analgesic activity is consistent with previous studies, such as those by Jeffrey Raj et al. (2020) and Bukhari et al. (2013), which also reported analgesic effects for piperine. The similarity in results further supports the hypothesis that piperine may be a promising candidate for developing novel analgesic agents targeting pain pathways.

4. Conclusion

This study highlights the therapeutic potential of piperine, a natural alkaloid derived from black pepper, in modulating inflammatory and nociceptive pathways. Efficient extraction methods, such as Soxhlet extraction,

facilitated the isolation of high-purity piperine, which was confirmed through comprehensive physicochemical and spectroscopic analyses.

In silico studies, including molecular docking, demonstrated that piperine exhibits a strong affinity for cyclooxygenase-2 (COX-2), with enhanced complex stability compared to the native ligand. These findings suggest that piperine may act as a potent COX-2 inhibitor, contributing to its anti-inflammatory effects. Furthermore, in vivo experiments revealed that piperine, at a dose of 50 mg/kg, showed anti-inflammatory activity comparable to aspirin and produced significant analgesic effects, confirming its potential as an effective therapeutic agent.

This research positions piperine as a promising candidate for developing novel anti-inflammatory and analgesic drugs. However, further studies are needed to elucidate its precise mechanisms of action, evaluate its long-term efficacy, and ensure its safety for clinical use.

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Conflict of interest

There is no conflict of interest.

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References

1. Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA. Biological role of Piper nigrum L. (Black pepper): A review. Asian Pacific Journal of Tropical Biomedicine. 1 janv 2012;2(3, Supplement):S1945-53.
2. Pei YQ. A review of pharmacology and clinical use of piperine and its derivatives. Epilepsia. avr 1983;24(2):177-82.
3. Eun JS. A Study on the Mechanism of Analgesic Action of Piperine. YAKHAK HOEJI. 30 août 1986;30(4):169-73.
4. Bukhari IA, Pivac N, Alhumayyd MS, Mahesar AL, Gilani AH. The analgesic and anticonvulsant effects of piperine in mice. J Physiol Pharmacol. déc 2013;64(6):789-94.
5. Vijayakumar RS, Surya D, Nalini N. Antioxidant efficacy of black pepper (Piper nigrum L.) and piperine in rats with high-fat diet induced oxidative stress. Redox Rep. 2004;9(2):105-10.
6. Kaur S, Sharma R, Sarangal V, Kaur N, Prashar P. Evaluation of anti-inflammatory effects of systemically administered curcumin, lycopene and piperine as an adjunct to scaling and root planing: A clinical study. Ayu. déc 2017;38(3-4):117-21.
7. De Cleyn R, Verzele M. Constituents of peppers. Chromatographia. 1 juill 1975;8(7):342-4.
8. Kannur D, Shingate PN, Dongre P. New method development for extraction and isolation of piperine from black pepper. International Journal of Pharmaceutical Sciences and Research. 1 janv 2013;4:3165-70.
9. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. J Comput Chem. déc 2009;30(16):2785-91.
10. Morris GM, Huey R, Olson AJ. Using AutoDock for Ligand-Receptor Docking. Current Protocols in Bioinformatics. 2008;24(1):8.14.1-8.14.40.
11. Castro-Alvarez A, Costa AM, Vilarrasa J. The Performance of Several Docking Programs at Reproducing Protein–Macrolide-Like Crystal Structures. Molecules. janv 2017;22(1):136.
12. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. Biophys Rev. 1 avr 2017;9(2):91-102.
13. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry. 1998;19(14):1639-62.
14. Rahmani S, Belboukhari N, Sekkoum K, Abdelkrim C. Evaluation DE L'ACTIVITÉ Anti-Inflammatoire D'extraits Aqueux DE Feuilles Limoniastrum feei (Plumbaginacea). Algerian Journal of arid environment. 1 juin 2016;6:80-6.
15. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. déc 1962;111:544-7.
16. Julien Wist. www.nmrdb.org Tools for NMR spectroscopists. [cité 15 août 2024]. NMR Predict. Disponible sur: https://www.nmrdb.org.