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## Variations in the Piperine content in three varieties of Pepper and mapping its anti-inflammatory potential by molecular docking

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**ABSTRACT:** Pepper known as the king of spices is one of the most widely used spice in the world, and it has its fair share of medicinal properties as well. It has been used in traditional medicine for many centuries owing to its medicinal properties apart from its flavour and pungent taste. Piperine is the most important bioactive compound present in different Piper species. Differences among the three Piper species common in South India viz. *Piper nigrum*, *Piper longum*, and *Piper cubeba* were evaluated by estimating the various phytochemicals such as alkaloids, flavonoids, and phenols in them. Further, the quantity of piperine in these three Piper species were determined using HPLC analysis. The piperine content in *Piper nigrum*, *Piper longum*, and *Piper cubeba* were found to be 235.05 µg/mL, 268.50 µg/mL, and 8.56 µg/mL respectively. Since inflammation is the major pathology involved in most of the disease conditions and since pepper is traditionally used in managing respiratory inflammation, we ventured to identify its anti-inflammatory potential. Herein, the anti-inflammatory properties of piperine were checked by in silico docking analysis of piperine with inflammatory proteins. The interaction of piperine with NF-κB, COX-2, COX-1, and TNF-α, as evidenced by the binding scores reveals that piperine interacts with them and modulates inflammation. Further *in-vitro* and *in-vivo* studies are warranted to scientifically validate the traditional claims.

## 1. INTRODUCTION

Spices were known for their medicinal value right after they were established for use in the society (Duke, 2002). India is known for a wide variety of spices due to its diverse climatic conditions. Amongst this pepper is prominent in India owing to its biodiversity. There are different species of pepper found in India such as *Piper nigrum* Linn. (black pepper), *Piper longum* Linn. (long pepper), *Piper cubeba* Linn. (tailed pepper) etc. all belonging to the family Piperaceae. *Piper nigrum* is native to Malabar coast of India as the vine requires tropical hot and humid climate with optimum temperature of 28°C and high rainfall of about 125-200cm for its growth (Prange, 2010). *Piper longum* is native to Indo-Malayan realm which includes different regions of India such as Kerala, Tamil Nadu, Assam, Madhya Pradesh, Meghalaya and many more. It requires a hot and humid climate, porous soil that is well drained and rich in organic matter with partial shade for its growth (Satyavati, 2021). *Piper cubeba* on the other hand is a native of Indonesia that also grows wild in the foothills of Himalayas in India, where it finds well drained soil with high humidity and optimum temperature (30-32°C) and rainfall (150cm).

Pepper has been widely used in AYUSH drug formulations (Ayurveda, Unani and Siddha medicine) due to its potential medicinal properties (Ahmad et al., 2012). *Piper longum* is the principal ingredient of some Ayurvedic medicines such as Trikatu churnam, Chavanprash and Amrit kalash. Trikatu churnam, a combination of *Piper nigrum* L. (black pepper), *Piper longum* L. (long pepper) and *Zingiber officinale* (ginger) is the major ingredient in more than 1500 Ayurvedic/Siddha formulations for treating various ailments (Committee, 1978). Trikatu is known to reduce Vata and Kapha and increase Pitta. Its Unani properties include control of temperament (Mizaj) (Kaushik et al., 2018). A range of biological activities exhibited is due to a wide spectrum of bioactive molecules present in the seeds, roots, stem and leaves of different pepper species (Mgbeahuruikwe et al., 2017). Medicinal use of a plant derived drugs in modern medicine is based on a single active compound in the purified form. Several bioactive compounds isolated and characterised from different varieties of pepper finds its use in various disorders afflicting humans. Herein, in the present study the bioactive molecule piperine in three Piper species commonly available in South India were determined using HPLC analysis. Further, since, inflammation is the major pathology involved in most of the disease conditions

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and since pepper is traditionally used in managing respiratory inflammation, we ventured to identify its anti-inflammatory potential by molecular docking.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

Analytical grade solvents from Sisco Research Laboratories (SRL), India, were used for phytochemical extraction. All other chemicals used were obtained from Himedia (India) and Sigma-Aldrich (St Louis, MO), unless otherwise specified.

### 2.2. Preparation of the extracts

Black pepper (*Piper nigrum*), long pepper (*Piper longum*) and tailed pepper (*Piper cubeba*) were purchased from Pondicherry market and they were verified by a botanist and voucher specimens were stored in the Natural Products Research Laboratory, Department of Biotechnology, Pondicherry University. Fifty gram of each sample was ground using mortar and pestle and then they were transferred to a conical flask and dissolved in 100% methanol. The conical flask was cotton plugged and covered using an aluminium foil to avoid oxidation by light and extracted by cold percolation method by shaking at 100 rpm for 48 hours. The supernatant was then collected in an amber coloured bottle using a Whatman filter paper. Methanol was removed using a rotary vacuum evaporator and the extracts were stored at 4°C until further use. The supernatant was then concentrated using a rotavapour at 30°C and 350 mbar. Concentrated sample was stored in a glass bottle at 4°C until further use.

### 2.3. Phytochemical Analysis of the Extracts

Preliminary qualitative phytochemical screening of the three varieties of the methanolic extract of the pepper species were checked for the presence of the primary and secondary metabolites such as: carbohydrates, proteins, steroids, glycosides, alkaloids, phenolics, flavonoids, tannins and saponins (Vasanthi et al., 2009). Further, the phenolic, flavonoid and alkaloid content were quantified spectrophotometrically (Harborne, 1984).

### 2.4. Chromatographic Analysis of the Pepper varieties

#### 2.4.1 High Performance Thin Layer Chromatography (HPTLC) fingerprinting of the pepper extracts

Fifty milligram of each pepper extract was dissolved in 1 mL of methanol by vortexing and filtered using a syringe filter. The precoated silica gel 60F<sub>254</sub> aluminium sheets (20 × 10 cm) used as stationary phase was activated in an oven at 50°C for 15 minutes. Toluene: ethyl acetate: formic acid (3:2:0.5 v/v/v) served as the mobile phase. The chromatographic conditions maintained includes: 30 min of plate and chamber saturation time; 70 mm of migration distance allowed; wavelength scanning done at 254 and 366 nm keeping the slit dimension at 10×0.6 mm. Ten microlitres of each of the extracts of *Piper longum*, *Piper nigrum*, *Piper cubeba* (5mg/ml) and the

phytoconstituents such as Piperine, Stigmasterol, Cinnamic acid, Eugenol, Ioeugenol, Quercetin and Gallic acid (1mg/mL) dissolved in methanol were spotted on the TLC plate and developed at constant temperature. Photometric measurements were performed at 254 and 366nm in reflectance mode using CAMAG-HPTLC (Switzerland) scanner and WINCATS software.

#### 2.4.2 High performance liquid chromatography (HPLC) quantification of piperine in the pepper extracts

The pepper extract was subjected to HPLC analysis by using Shimadzu UFLC equipped with UV detector. Quantification of Piperine in *Piper longum*, *Piper nigrum* and *Piper cubeba* against the standard Piperine was performed by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) following the method of Upadhyay et al. (2013). C18-250X4.60 mm 5 μm, 100 Å column was used to separate piperine. Acetonitrile and HPLC grade water were used as the mobile phase in the ratio (60:40). Isocratic elution was performed with 60% of acetonitrile with a run time of 30 minutes. A flow rate of 1 ml/min was preserved throughout the run and the phytomolecule was detected using an UV-detector at 254 nm. The standard concentration of piperine was made at 2 mg/mL and its injection volume was kept at 25 μL. The sample concentration of *Piper nigrum* and *Piper longum* were made at 62.5 ng/mL and for *Piper cubeba*, it was made at 250 ng/mL. The injection volume for all the pepper varieties were kept at 25 μL. Temperature was maintained at 25°C.

### 2.5. In silico Docking Analysis

#### 2.6. Protein Preparation

Four target proteins viz., TNFα, NF-κB, COX-1, and COX-2 that are well known for mediating inflammatory response were selected for the present study. Three-dimensional (3D) conformers of the target proteins of interest TNFα (PDB code: 2AZ5), NF-κB (PDB code: 3GUT), COX-1 (PDB code: 5WBE) and COX-2 (PDB code: 1CX2) were downloaded from www.rcsb.org as Protein Data Bank (PDB) format, one of the primary archival resources in areas of structural biology for further *in-silico* docking analysis (Burley et al., 2019). Using PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), all the relevant literatures required for the present study were collected. All protein structures were visualized in PYMOL to check for structural integrity and unwanted amino acid chains were removed to avoid overlapping results derived from homo, di / tri / tetramer proteins. Polar hydrogens were then added to the structure in Auto dock tools along with addition of Kollman charges. Atoms were further assigned as AD4 type and the PDB file was saved in .pdbqt format. The proteins such as TNFα (PDB code: 2AZ5), NF-κB (PDB code: 3GUT), COX-1 (PDB code: 5WBE) and COX-2 (PDB code: 1CX2) were then analysed by checking the intactness of proteins and by removing the extra chains associated with the protein. Simultaneously, polar hydrogen bonds were added to the protein molecules along with Kollman charges. Then the proteins were assigned

AD4 type atoms to strengthen the molecule. All the files were then saved in pdbqt format to prepare them for interacting with the ligands of interest.

## 2.7. Ligand Preparation

The bioactive phytochemical in pepper which is piperine was the ligand of interest to carry out this study. Piperine was chosen as it is the main bioactive compound in pepper and known for its pharmacological effects (Derosa et al., 2016; Gorgani et al., 2017). Accordingly, the structures of the ligand were drawn using ChemBioDraw, and the 2D conformation of the ligand was subjected to 3D-optimisation by ChemSketch, one of the best chemical drawing software and was converted to 3D conformer. Subsequently, Chimera software was used for converting ligand and protein files from .sdf to .pdb format. Ligands (.pdb) were opened in autodock tools and were saved in.pdbqt format after detecting the route.

## 2.8. Docking

Auto dock tools (ADT) v1.5.6 and Autodock v4.2 programs were used to carry out docking analysis (Morris et al., 2009). To run Auto dock 1.5.6, a searching grid is generally used which would be extended over the selected amino acids in the receptor protein along with the addition of polar hydrogens to the ligand moieties, atomic salvation parameters and assignment of Kollman charges. Gasteiger-type polar hydrogen charges were assigned and carbons were merged with the nonpolar hydrogens and the internal degrees of freedom and torsions were established. Thereafter, grid parameter files were saved in .gpf format followed by autogrid run. Computational binding or docking took place in between the bioactive molecule piperine and the respective receptor proteins wherein the ligands were considered as flexible and the macromolecule was regarded as a rigid body. Lamarckian Genetic Algorithm was applied to carry out the search and 20 runs were performed for this study. Gromos force field was used to minimize energy. The whole receptor protein was used for blind docking. Affinity maps for all the atom types present, as well as an electrostatic map were computed with a grid spacing of 0.375 Å. Search and docking parameters were selected before running the final auto dock. After accepting docking parameters, output files were saved as .dpf (docking parameter file) format followed by selection of program path name, parameter file and autodock was run. After completion of docking, the resulted files were saved as .dlg (docking log file) format. The final projection of the interaction and docking pose analysis was done by LigPlus, a software used to visualize the environment of a ligand either by downloading from the PDB ligand-protein complex or by extracting a docked pose from a docking run complexed with the target protein. To perform this, .dlg file was opened in auto dock and different docking pose conformation were analysed using conformation play option in auto dock and hydrogen bonds were also built. The docked protein- ligand complex was then saved as .pdb format which was further analysed using LigPlot+ tool. Binding energies of different generated protein-ligand complex were also

recorded by LigPlus. Finally, the final .pdb complex was run using LigPlus to show the active amino acid sites and hydrogen bonds between the protein and ligand. The different complexes were then sorted with respect to the predicted binding energy and analysed.

## 2.9. Statistical Analysis

Results of phytochemical analysis are expressed as mean  $\pm$  SD based on triplicate analysis performed.

## 3. RESULTS AND DISCUSSION

Pepper has been used for centuries for its culinary and medicinal properties. It has been used in Ayurveda, Siddha, and traditional Chinese medicine due to the wide range of bio-activities they exhibit such as anti-microbial, anti-diabetic, anti-inflammatory, gastro-intestinal stimulant, anti-asthmatic, anti-carcinogenic, anti-hyperlipidemic, anti-diarrheal and anti-oxidant activities (Saxena et al., 2022). Further, it is well known as a bioenhancer in most AYUSH preparations and is now being used in developing nutraceutical supplements/formulations enhancing the bioactivity of some lead molecules. The reason behind their diverse pharmacological activity is the presence of multiple bioactive compounds in pepper. However, to further refine and understand the intricacies of their mechanism, an in-depth study of their bioactive molecules is required. In the present study the variations in the phytochemical constituents in three different varieties of pepper commonly available in South India were analysed and the bioactive molecule piperine was quantified and checked for its anti-inflammatory potential using *in-silico* techniques.

### 3.1. Phytochemical profiling of the Pepper Extracts

The phytochemicals in various medicinal plants have been studied extensively for their role in preventing disease and promoting health by establishing their efficacy and understanding their probable mode of action (Sangeetha et al., 2013; Sundaram et al., 2019a). Accordingly, the preliminary qualitative phytochemical analysis revealed the presence of the primary metabolites such as carbohydrates and reducing sugars in the form of glycosides, amino acids and proteins and steroids. The secondary metabolites include the polyphenols, flavonoids, saponins and alkaloids. It was observed that all the three pepper varieties showed consistent results throughout the test with only slight variation in the final colour intensities in the respective tests; *Piper nigrum* showing the highest intensity followed by *Piper longum* and the least intense being *Piper cubeba* (Table 1). The variation in the intensity owes to the presence of different levels of phytochemicals in the different species of the genus piper. Therefore, we further quantified the total phenolic content, flavonoids and total alkaloid content spectrophotometrically. It is interesting to note that although the polyphenols and flavonoids in general exhibit various pharmacological potential, the alkaloid content was higher than the polyphenols and flavonoids in all the three species (Table 2). Among the three species tested, the alkaloid content was found

**Table 1**  
Qualitative analysis of the pepper varieties

Test	Colour Observed	<i>P. longum</i>	<i>P. nigrum</i>	<i>P. cubeba</i>
<b>Protein:</b>				
Biuret test Millon's test	Red or violet Red ppt	- ++	- +	- +
<b>Steroid:</b>				
Salkowski test	Brown ring	-	-	-
<b>Carbohydrate:</b>				
Molisch test	Violet ring	+	+	+
<b>Reducing sugar:</b>				
Fehling's test Benedict's test	Brick red ppt Green, yellow or red colour	+++ +++	+++ ++	++ ++
<b>Glycosides:</b>				
Anthrone test	Dark green colour	+	+	+
<b>Phenol:</b>				
Ferric chloride test	Bluish green	+	+++	+++
<b>Tannins:</b>				
Lead acetate test	Orange red ppt	+	+++	+
<b>Flavonoids:</b>				
Shinoda test Alkaline reagent	Red colour Dark yellow colour	- ++	- +	- +
<b>Saponins:</b>				
Foam test	Persistent foam	++	+	+
<b>Alkaloids:</b>				
Dragendroff Mayer's test Wagner's test	Orange red ppt Creamy white ppt Reddish brown ppt	++- +++	+++ - ++	- + -

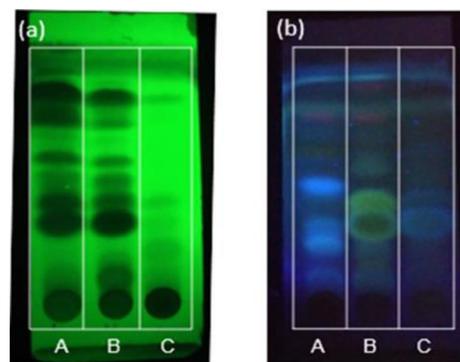
The symbols used in the table denotes: - absent, + mildly present, ++ moderately present, +++ highly present.

to be maximum in *Piper longum* (1.478 mg/g) followed by *Piper nigrum* (0.601 mg/g) and *Piper cubeba* (0.316 mg/g). It has been reported that the presence of alkaloids could express many pharmacological activities such as antihypertensive effects, antiarrhythmic effects, and anticancer actions (Alencar et al., 2007). The above results confirms that there is variation in the secondary metabolite content in the three pepper varieties tested. The differences identified is not only because they belong to different species but also due to the difference in the geographical and climatic conditions where they grow (Zou & Zou, 2021).

Subsequently, the TLC elution profiles of the three pepper extracts viz. *Piper longum*, *Piper nigrum*, and *Piper cubeba* were visualized under near UV (254 nm) & far UV (366 nm) (Figure 1). At least 15 distinguishable bands were observed for both *Piper longum* and *Piper nigrum* whereas *Piper cubeba* showed only 8 such distinguishable bands. It was also interesting to note that the intensity of the bands was quite high for both *Piper longum* and *Piper nigrum* whereas *Piper cubeba* showed faint bands. This result concluded that *Piper nigrum* and *Piper longum* have a variety of secondary metabolites in higher concentration as compared to *Piper cubeba* which gave few distinguishable bands of low intensity.

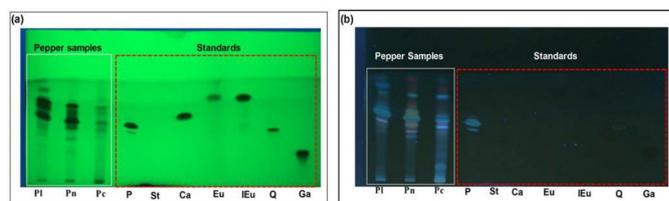
Further, as TLC is a crude method used for initial band visualization, High-performance thin-layer chromatography (HPTLC) profiling provided a much more refined and detailed analysis.

The relative front values of the individual bands in each of the extract lanes viz. *Piper longum* (Pl); 5 mg/ml, *Piper nigrum* (Pn); 5 mg/ml and *Piper cubeba* (Pc); 5 mg/ml were matched



**Figure 1.** TLC chromatogram of pepper varieties. (a) 254 nm (b) 366 nm

A, B and C corresponds to *Peper longum*, *Piper nigrum* and *Piper cubeba*



**Figure 2.** HPTLC chromatogram of pepper varieties (a) 254 nm (b) 366 nm  
Pl, Pn, Pc, P, St, Ca, Eu, Ieu, Q, Ga corresponds to *Piper longum*, *Piper nigrum*, *Piper cubeba*, Piperine, Stigmasterol, Cinnamic acid, Eugenol, Isoeugenol, Quercetin and Gallic acid respectively.

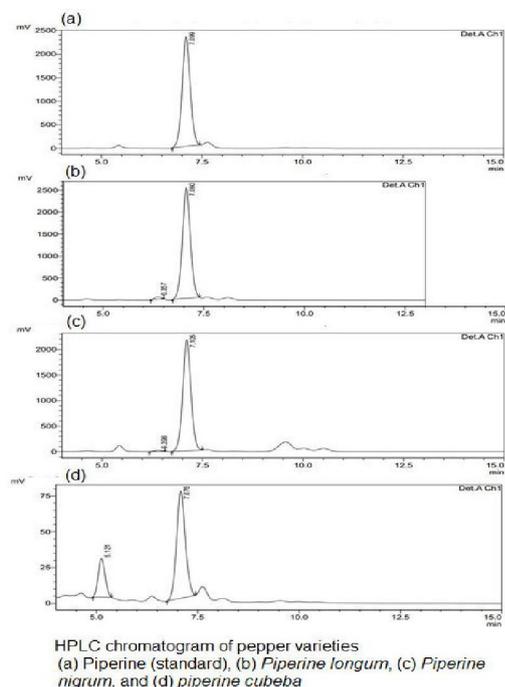
**Figure 2.** HPTLC chromatogram of pepper varieties.

against phytochemical standards viz., Piperine (P); 1 mg/ml, Stigmasterol (St); 1 mg/ml, Cinnamic acid (Ca); 1 mg/ml, Eugenol (Eu); 5 mg/ml, Isoeugenol (IEu); 1 mg/ml, Quercetin (Q); 1 mg/ml and Gallic acid (Ga); 1 mg/ml were visualized in both near UV (254 nm) and far UV (366 nm) (Figure 2).

**Table 2**  
Quantitative analysis of the pepper varieties

Phytochemicals	Pepper species		
	<i>Piper longum</i> (mg/g)	<i>Piper nigrum</i> (mg/g)	<i>Piper cubeba</i> (mg/g)
Alkaloids	1.478±0.02	0.601±0.03	0.316±0.02
Phenols	0.065±0.02	0.164±0.02	0.116±0.02
Flavonoids	0.021±0.01	0.020±0.01	0.012±0.01

It was observed that only the standards - Piperine, Eugenol, Isoeugenol, and Quercetin matched against their respective bands present in the extracts. Among the three extracts, *Piper cubeba* showed the least intense bands when compared with *Piper longum* and *Piper nigrum*. The results implied that the current solvent system was able to simultaneously elute and resolve discrete bands of only Piperine, Eugenol, Isoeugenol, and Quercetin along with *Piper longum*, *Piper nigrum*, and *Piper cubeba*. Also, from the intensity of the bands in the three *Piper* extracts, it was concluded that the individual components present in them were in a higher concentration in *Piper longum* and *Piper nigrum* but not for *Piper cubeba* which was noticed in our TLC analysis also.



**Figure 3.** HPLC chromatogram of pepper varieties.

It is well known that high pressure liquid chromatography (HPLC) is more sensitive than any other analytical technique for qualitative and quantitative analysis of various compounds (Blum, 2014). However, method development and optimization of sensitivity to detect analytes in various samples is still a challenge (Upadhyay et al., 2013). In the present study, the total run time of piperine was found to be 15 minutes, and the piperine appeared on chromatogram at 7.105 minutes for *Piper nigrum* extract, at 7.060 minutes for *Piper longum*

extract, at 7.076 minutes for *Piper cubeba* extract (Figure 3). The retention time of the reference standard, piperine (Figure 3) was observed at 7.099 minutes. It was also noted that apart from the piperine peaks all the pepper extracts showed small peaks; *Piper nigrum* showed a small peak at 6.398 minutes, *Piper longum* showed a small peak at 6.357 minutes and *Piper cubeba* showed a small peak at 5.121 minutes. The amount of piperine present in the three varieties of pepper is computed in (Table 3). The results confirm that the current HPLC method was a stable, convenient, and rapid method for determining the purity and concentration of piperine. Throughout the experiment chromatographic traces were completely clean and none exhibited any type of interference throughout the entire running time confirming the sensitivity and specificity of the method used in quantification.

Modern medicine is based upon several parameters such as identification and isolation of a particular molecule and its subsequent synthetic modification to achieve a greater degree of efficacy or potency which includes its dosage and toxicity profiling before it reaches the market. Piperine or piperoylpiperidine constitutes about 98% of the total alkaloid found in pepper, it is hydrophobic and interacts with different cellular proteins and factors to either inhibit them or activate them (Meghwal & Goswami, 2012). It also has bio-enhancing activity wherein, it increases the efficacy of certain drugs by increasing its bioavailability either by altering the membrane dynamics, or inhibiting the gastrointestinal and hepatic metabolism (Peterson et al., 2019). The amount of piperine present in *Piper nigrum* was 235.05 µg/mL and *Piper longum* accounted for a piperine content of 268.50 µg/mL whereas, *Piper cubeba* possessed only 8.56 µg/mL of piperine. Our results corroborate to an earlier report on the piperine content in black pepper (Hamrapurkar et al., 2011). Piperine exists in three more isomeric states namely isopiperine (cis–trans isomer), chavicine (cis–cis isomer), and isochavicine (trans–cis isomer) of which only the former is known to contribute to the pungency of black pepper. Apart from the piperine peaks, all the pepper extracts showed small peaks with different retention times which is probably due to the isomeric forms or other piperine derivatives such as piperamide, piperamine, etc.

### 3.2. In silico Docking Analysis:

The present work aimed to evaluate the role of piperine on target proteins that modulate inflammation as it is one of the key pathologies involved in most chronic disorders and since pepper is traditionally used in managing respiratory inflammation (Rehman et al., 2015). The inflammatory

**Table 3**  
Quantification of piperine content in the pepper varieties

<i>Piper longum</i>				
Sample	PL 5.1	PL 5.2	PL 5.3	Average
Peak area	35643748	35136141	34939261	35239716.67
Amount of piperine [ $\mu\text{g/ml}$ ]	271.58	267.71	266.21	268.50
<i>Piper nigrum</i>				
Sample	PN 4.1	PN 4.2	PN 4.3	Average
Peak area	31084153	31413053	30050962	30849389.33
Amount of piperine [ $\mu\text{g/ml}$ ]	236.84	239.34	228.96	235.05
<i>Piper cubeba</i>				
Sample	PLC 3.1	PLC 3.2	PLC 3.3	Average
Peak area	1145074	1144369	1081919	1123787.33
Amount of piperine [ $\mu\text{g/ml}$ ]	8.72	8.71	8.24	8.56

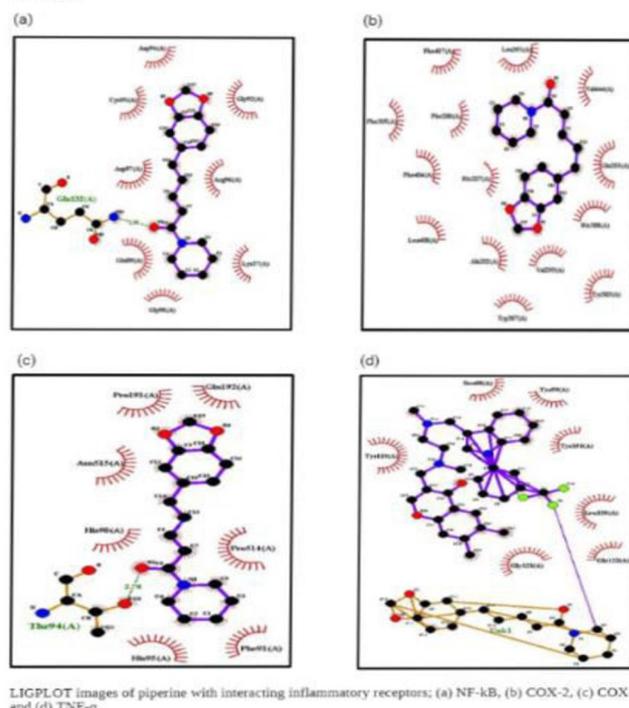
response is associated with the expression of several proteins such as *NF- $\kappa$ B*, COX-2, COX-1, and TNF- $\alpha$  (Sundaram et al., 2019b). Therefore, the influence of piperine on *NF- $\kappa$ B*, COX-2, COX-1, and TNF- $\alpha$  was evaluated using in-silico analysis. Several scientific studies have shown that COX has two isoforms responsible for the synthesis of prostaglandins among which COX-1 derived prostaglandins mainly mediates cytoprotective actions on the gastrointestinal mucosa whereas Cyclooxygenase-2 (COX 2) is responsible for the production of inflammatory mediators such as prostaglandins and its metabolites which are involved in vasodilation, thermoregulation, etc. (Zarghi & Arfaei, 2011); whereas *NF- $\kappa$ B* can induce the expression of pro-inflammatory cytokines, chemokines, and genes involved in inflammasome complex formation (Dorrington & Fraser, 2019). On the other hand, TNF- $\alpha$  is a multifunctional cytokine involved in systematic inflammation and is one of the cytokines that make up the acute phase reaction and their respective metabolites (Parameswaran & Patial, 2010).

**Table 4**

Ligand and inflammatory receptors with their binding scores and interacting amino acid.

Receptor	Ligand (piperine)	Interacting amino acid
<i>NF-<math>\kappa</math>B</i>	-2.68	(H-Glutamine 132)
COX-2	-5.33	(Hydrophobic interaction)
COX-1	-3.0	(H-Threonine 94)
TNF- $\alpha$	-4.07	(H-Serine 95)

Docking studies are important for predicting the functionality of the bioactive compounds before conducting wet-lab experiments and most frequently it is an important aspect in structure-based drug designing (Ferreira et al., 2015). Docking simulation of the alkaloid piperine with the ligands (inflammatory markers) viz. *NF- $\kappa$ B*, COX-2, COX-1, and TNF- $\alpha$  were performed and the binding conformations and binding affinities were obtained by using the docking protocol, Autodock (autodoc tools- 1.5.4 version). All the possible rotatable bonds of the compound piperine were taken into account in the process of docking to identify the best binding confirmation with the above-mentioned inflammatory



**Figure 4.** LIGPLOT images of piperine with interacting inflammatory receptors.

proteins. *NF- $\kappa$ B* showed the binding score of -2.68 in the conformation where piperine bonded with Glutamine-132 via hydrogen bond in the binding pocket of the former protein whereas COX-2 showed the best binding score of -5.33 in the conformation mentioned in the figure where piperine hydrophobically interacted with the protein molecule. On the other hand, COX-1 showed the best binding score of -3.0 in the conformation where piperine bonded with Threonine 94 via hydrogen bond whereas TNF- $\alpha$  showed the best binding score of -4.07 in the conformation where piperine bonded with Serine 95 via hydrogen bond (Table 4) and (Figure 4). This proves that piperine has a significant binding interaction with these inflammatory proteins thereby modulating their expression. In the present study, molecular docking studies of

inflammatory marker proteins with piperine provides valuable insights about binding stability and scoring functions with the targeted receptors. Our results are in congruence with the earlier studies on the anti-inflammatory potential of piperine on fibroblast cell lines (Bang et al., 2009), and in experimental animal models of inflammation wherein, piperine significantly reduced the proinflammatory mediator TNF  $\alpha$  in experimental rats (Umar et al., 2013). However, further studies are warranted to validate the traditional claims and identify the detailed mode of action of piperine before translating it to clinical use.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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## AUTHOR CONTRIBUTIONS

JH and VKS carried out the experimental work and compiled the results and wrote the manuscript and HRV designed the work and edited the manuscript.

## REFERENCES

- Ahmad, N., Fazal, H., Abbasi, B.H., Farooq, S., Ali, M., Khan, M.A., 2012. Biological role of *Piper nigrum* L. (Black pepper): A review. *Asian Pacific Journal of Tropical Biomedicine*. 2(3), S1945–S1953. [https://doi.org/10.1016/S2221-1691\(12\)60524-3](https://doi.org/10.1016/S2221-1691(12)60524-3)
- Alencar, S.M., Oldoni, T., Castro, M.L., 2007. Chemical composition and biological activity of a new type of Brazilian propolis: red propolis. *Journal of Ethnopharmacology*. 113(2), 278–283. <https://doi.org/10.1016/j.jep.2007.06.005>
- Bang, J.S., Oh, D.H., Choi, H.M., 2009. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1beta-stimulated fibroblast-like synoviocytes and in rat arthritis models. *Arthritis Research & Therapy*. 11(2), R49. <https://doi.org/10.1186/ar2662>
- Blum, F., 2014. High performance liquid chromatography. *British Journal of Hospital Medicine*. 75(2), C18–C21. <https://doi.org/10.12968/hmed.2014.75.sup2.c18>
- Burley, S.K., Berman, H.M., Bhikadiya, C., et al., 2019. RCSB Protein Data Bank: Biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. *Nucleic Acids Research*. 47, D464–D474. <https://doi.org/10.1093/nar/gky1004>
- Committee, A.P., 1978. *The Ayurvedic Formulary of India*, Part 2.
- Derosa, G., Maffioli, P., Sahebkar, A., 2016. Piperine and Its Role in Chronic Diseases, and others, (Eds.), *Anti-inflammatory Nutraceuticals and Chronic Diseases*. Springer, Cham, pp. 173–184.

- [https://doi.org/10.1007/978-3-319-41334-1\\_8](https://doi.org/10.1007/978-3-319-41334-1_8)
- Dorrington, M.G., Fraser, I., 2019. NF- $\kappa$ B Signaling in Macrophages: Dynamics, Crosstalk, and Signal Integration. *Frontiers in Immunology*. 10, 705. <https://doi.org/10.3389/fimmu.2019.00705>
- Duke, J.A., 2002. CRC Handbook of Medicinal Spices, In: 1st (Eds.); and others, (Eds.). CRC Press, Boca Raton, p. 360. <https://doi.org/10.1201/9781420040487>
- Ferreira, L.G., Santos, R.N., Oliva, G., Andricopulo, A.D., 2015. Molecular docking and structure-based drug design strategies. *Molecules*. 20(7), 13384–13421. <https://doi.org/10.3390/molecules200713384>
- Gorgani, L., Mohammadi, M., Najafpour, G.D., Nikzad, M., 2017. Piperine-The Bioactive Compound of Black Pepper: From Isolation to Medicinal Formulations. *Comprehensive Reviews in Food Science and Food Safety*. 16(1), 124–140. <https://doi.org/10.1111/1541-4337.12246>
- Hamrapurkar, P.D., Jadhav, K., Kundnani, P., Zine, S., 2011. Quantitative Estimation of Piperine in *Piper nigrum* and *Piper longum* Using High Performance Thin Layer Chromatography. *Journal of Applied Pharmaceutical Science*. 3, 117–120.
- Harborne, J.B., 1984. *Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis*, and others, (Eds.). Springer Dordrecht, p. 288. <https://doi.org/10.1007/978-94-009-5570-7>
- Kaushik, R., Jain, J., Khan, D., Rai, P., 2018. Trikatu - A combination of three bioavailability enhancers. *International Journal of Green Pharmacy*. 12, S437. S441
- Meghwal, M., Goswami, T.K., 2012. Nutritional Constituent of Black Pepper as Medicinal Molecules: A Review. *Open Access Scientific Reports*. 1, 129. <http://dx.doi.org/10.4172/scientificreports.129>
- Mgbeahuruike, E.E., Yrjönen, T., Vuorela, H., Holm, Y., 2017. Bioactive compounds from medicinal plants: Focus on *Piper* species. *South African Journal of Botany*. 112, 54–69. <https://doi.org/10.1016/j.sajb.2017.05.007>
- Morris, G.M., Ruth, H., Lindstrom, W., 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*. 30(16), 2785–2791. <https://doi.org/10.1002/jcc.21256>
- Parameswaran, N., Patial, S., 2010. Tumor necrosis factor- $\alpha$  signaling in macrophages. *Critical Reviews in Eukaryotic Gene Expression*. 20(2), 87–103. <https://doi.org/10.1615/critrevueukargeneexpr.v20.i2.10>
- Peterson, B., Weyers, M., Steenekamp, J.H., Steyn, J.D., Gouws, C., Hamman, J.H., 2019. Drug Bioavailability Enhancing Agents of Natural Origin (Bioenhancers) that Modulate Drug Membrane Permeation and Pre-Systemic Metabolism. *Pharmaceutics*. 11(1), 33. <https://doi.org/10.3390/pharmaceutics11010033>
- Prange, S.R., 2010. 'Measuring by the bushel': reweighing the Indian Ocean pepper trade. *Historical Research*. 84(224), 212–235. <https://doi.org/10.1111/j.1468-2281.2010.00547.x>
- Rehman, A., Mehmood, M.H., Haneef, M., 2015. Potential of black pepper as a functional food for treatment of airways disorders. *Journal of Functional Foods*. 19, 126–140. <https://doi.org/10.1016/j.jff.2015.09.006>
- Sangeetha, M.K., Priya, C., Vasanthi, H.R., 2013. Anti-diabetic property of *Tinospora cordifolia* and its active compound is mediated through the expression of Glut-4 in L6 myotubes. *Phytomedicine*. 20(3–4), 246–248. <https://doi.org/10.1016/j.phymed.2012.11.006>
- Satyavati, G., 2021. Medicinal plants of India. [https://scholar.google.co.in/scholar?hl=en&as\\_sdt=0%2C5&q=3.%09Satyavati+G.+Piper+Linn.+Medicinal+plants+of+India.+1987%3B2%3A426-456.&btnG=&.Date+accessed:+2021-06-03](https://scholar.google.co.in/scholar?hl=en&as_sdt=0%2C5&q=3.%09Satyavati+G.+Piper+Linn.+Medicinal+plants+of+India.+1987%3B2%3A426-456.&btnG=&.Date+accessed:+2021-06-03)
- Saxena, M., Saxena, J., Nema, R., Singh, D., Gupta, A., 2022. Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry*. 1(6), 168–182.

- Sundaram, L., Vasanthi, R., R, H., 2019a. Dalspinin isolated from *Spermacoce hispida* (Linn.) protects H9c2 cardiomyocytes from hypoxic injury by modulating oxidative stress and apoptosis. Journal of Ethnopharmacology, 241–241. <https://doi.org/10.1016/j.jep.2019.111962>
- Sundaram, L., Vasanthi, R., R, H., 2019b. Dalspinin isolated from *Spermacoce hispida* (Linn.) protects H9c2 cardiomyocytes from hypoxic injury by modulating oxidative stress and apoptosis. Journal of Ethnopharmacology, 241–241. <https://doi.org/10.1016/j.jep.2019.111962>
- Umar, S., Sarwar, G., Umar, A., K., 2013. Piperine ameliorates oxidative stress, inflammation and histological outcome in collagen induced arthritis. Cellular Immunology. 284(1-2), 51–59. <https://doi.org/10.1016/j.cellimm.2013.07.004>
- Upadhyay, V., Sharma, N., Joshi, H.M., 2013. Development and Validation of Rapid RP-HPLC Method for Estimation of Piperine in *Piper nigrum* L. ~ 6 ~. International Journal of Herbal Medicine. 1(4), 6–9.
- Vasanthi, H.R., Mukherjee, S., Lekli, I., Ray, D., Veeraraghavan, G., Das, D.K., 2009. Potential role of *Borreria hispida* in ameliorating cardiovascular risk factors. Journal of Cardiovascular Pharmacology. 53(6), 499–506. <https://doi.org/10.1097/fjc.0b013e3181a7b677>
- Zarghi, A., Arfaei, S., 2011. Selective COX-2 Inhibitors: A Review of Their Structure-Activity Relationships. Iranian Journal of Pharmaceutical Research : IJPR. 10(4), 655–655.
- Zou, Z., Zou, X., 2021. Geographical and Ecological Differences in Pepper Cultivation and Consumption in China. Frontiers in Nutrition. 8, 718517. <https://doi.org/10.3389/fnut.2021.718517>