RESEARCH PAPER

Phytochemical Screening, Characterization, and in vitro Anti-Inflammatory Evaluation of Piperine from Piper nigrum Linn.

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ABSTRACT
Black pepper is internationally recognized as a distinctive flavour enhancer. It is often referred to as the "King of spices" by numerous individuals. The robust and spicy flavour is attributed to a compound known as piperine. Recent studies have indicated that an initial analysis of piperine showed the existence of flavonoids, phenols, and carbohydrates. This research indicates that the thin-layer chromatography Rf value recorded is 0.4. The subsequent mass analysis indicated Peak 1 at 268.1905, and the infrared (IR) spectroscopic evaluation of piperine showed a peak at 1579. 70. Additionally, piperine has demonstrated the ability to reduce inflammation. The albumin denaturation assay was utilized to evaluate the in vitro anti-inflammatory properties. At extract with an IC50 value of 88.34 mg/ml and compound with an IC50 value of 128.47 mg/ml while the standard diclofenac sodium had an IC50 value of 121.29 mg/ml. In conclusion, piperine extract and compound displayed good anti-inflammatory properties.

Keywords: Piperine, Phytochemical, Thin layer chromatography and anti-inflammatory
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INTRODUCTION

Among all the different spices we can find, black pepper is a well-known and special taste enhancer all over the world. In fact, many people call it the "King of spices". Its strong and spicy taste comes from a substance called piperine (Chopra B, et al 2016). How much piperine there is can depend on things like the weather, how it's dried, and where it comes from (Sozzi GO, et al 2012). Piperine, known as the main thing that makes black pepper spicy, was first taken out in 1819 by Hans Christian Ørsted. He separated a yellowish, crystal-like substance, with the chemical formula C17H19NO3 that melts when it reaches 128-130 º C. Later on, the way the compound was put together was figured out, and its IUPAC name is (2E, 4E)-5-(benzo[d] [1,3]dioxol-5-yl)1-(piperidin-1-yl)penta-2,4-dien-1-one. Piperine isn't very basic and can be broken down by hydrolysis (using acid or base) into piperic acid and piperidine (Pruthi JS. Et al 1999). There is a structure made of linked aliphatic molecules that acts like a bridge connecting piperidine with the 5-(3,4 methylenedioxyphenyl) part. Because of this, piperine is a really special molecule that can easily attach to CYP-450 enzymes. Black pepper has four different types of piperine that are isomers, which are called trans-trans isomer (1, piperine), cis-trans isomer (2, isopiperine), cis-cis isomer (chavicine, 3) and trans-cis isomer (isochavicine, 4). The geometrical isomers are not as spicy as piperine (Ravindran P. 2003). There are also other alkaloids in black pepper extracts, like piperanine, 5, piperettine, 6, piperylin A, 7, piperolein B, 8, and pipericine, 9 (Hirasa K, et al 1998). Isomerization is more likely to happen when there is a lot of light for a long time. Light can cause piperine to change into isopiperine2, chavicine 3, and isochavicine. You can see chavicine changing into piperine when it's being stored, which makes it gradually become less spicy over time (Kozukue N, et al 2007) People often use piperine in traditional medicine in Chinese and Indian practices. It's used for many things, like managing pain, relieving chills, dealing with rheumatism arthritis, fighting the flu, and lowering fever (Zachariah TJ, et al 2007). Piperine has also been shown to help improve blood flow, make you produce more saliva, and increase your appetite (Pruthi JS. Et al 2004). Piperine has been shown to have a wide array of effects on the body, such as alleviating pain, lowering blood pressure, modulating blood vessel cells (Hlavačková L et al 2011), and working against cancer. Moreover, it has an effect on several enzyme systems (including p-glycoproteins) (Li S, et al 2011). Piperine has been found to perform a number of biological functions, for example, combating infections, eliminating microbes, eradicating insects, decreasing inflammation, combating amoeba, healing sores, and improving one's mood (Zarai Z, et al 2013). Piperine enhances the rate at which various drugs are absorbed and their availability within the body (Miyakado M, et al 1979 & Lee SA, et al 2005). Rajendra Prasad et al. emphasized piperine's ability to increase the bioavailability of medications for tuberculosis. Furthermore, piperine has been shown to have a number of pharmacological advantages by altering the actions of transporters and metabolic enzymes. Furthermore, it is suggested that piperine could be used as an alternative drug. Park et al. reported on the effect of piperine on 3T3-L1 cell lines, where it suppressed the production of PPAR-γ, and is therefore helpful in the treatment of

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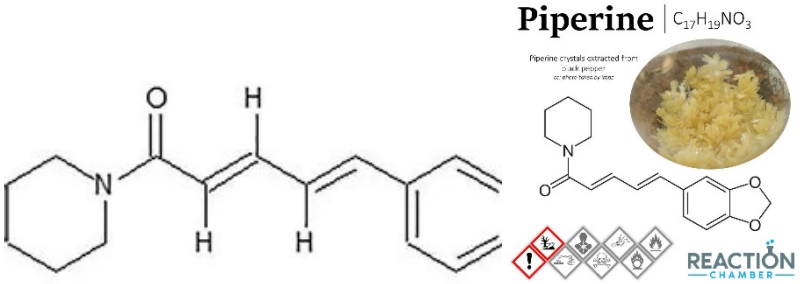
conditions related to obesity. Ahmad et al. covered the biological benefits and diverse biological activities of pipernigrum, the impact of piperine on digestion, its antioxidant properties, and its role in the treatment of various ailments. Present work is to isolation and characterization of the compounds piperine from pepper and investigated for invitro anti-inflammatory activity for extract and isolated compound to explore anti-inflammatory potential.

Structure:
The molecular formula of piperine is $C_{17}H_{19}NO_3$ and its molecular structure



PIPERINE –Structure

MATERIAL AND METHODS

Collection of samples:
Pepper powder collected from Aravindh herbal labs Rajapalayam, Srivilliputhur, Tamilnadu for this investigation.

Method of preparation of samples:
10 gram of pepper powder is heated with 100 ml of ethanol separately for 5 hours under soxhlet apparatus with condenser by hot continuous hot extraction method in water bath, cool and filter. The filtrate is evaporated under vacuum to get ethanol extract.

ISOLATION OF PIPERINE

To isolate piperine from black pepper (*Piper nigrum*), the process typically begins with solvent extraction using a Soxhlet apparatus or simple reflux. Finely ground black pepper is treated with 95% ethanol or dichloromethane and heated for several hours. This stage extracts the alkaloids, resins, and volatile oils from the plant matrix. Once the extraction is complete, the solution is filtered and concentrated under reduced pressure using a rotary evaporator to yield a thick, dark brown oleoresin. To separate the piperine from the unwanted acidic resins, the residue is treated with an alcoholic potassium hydroxide (KOH) solution. This step is crucial as it saponifies the resins, making them soluble in the aqueous phase while the piperine remains insoluble. The mixture is filtered, and the resulting filtrate is allowed to stand overnight. As the solution cools or is diluted with water, piperine begins to precipitate out as crude yellow crystals. These crystals are collected via vacuum filtration. To achieve high purity, the crude product is recrystallized using hot ethanol. The final result is needle-like, pale yellow crystals of piperine with a characteristic melting point between 128°C and 130°C (Stahl E, et al, 1981).

METHODOLOGY

PHYTOCHEMICAL ANALYSIS

Phytochemical screening is the process used to identify various compounds found in plant extracts. Plants contain numerous chemical constituents that can elicit different physiological responses and offer therapeutic advantages. Consequently, it is standard practice to evaluate plants for the existence of biologically active

and medicinally significant phytochemicals. Constituents responsible for a particular biological activity. Some of the examples of phytoconstituents include alkaloids, steroids, carbohydrates, saponins, tannins, flavonoids etc (Farnsworth NR. et al 1966 & Haseen, A., et al 2024).

THIN LAYER CHROMATOGRAPHY

10 ml of the methanol was taken as a mobile phase in the three different small TLC Chamber. The chamber was sealed and permitted to reach saturation for 30 minutes. The 10cm length 3.3 cm breath TLC glass plate was taken. The TLC plate was prepared by pouring method from the slurry of the Silica gel G and distilled water. Following the preparation of the TLC plate, it was placed in a hot air oven for 30 minutes at 105°C to eliminate any moisture in the stationary phase. The eluted compounds were taken in the three separate capillary tubes and applied as a small spot on the three separate TLC plate at 2cm above from the base of the plate and allowed to dry in the air. The three TLC plates were placed inside three separate small TLC chambers containing methanol as the mobile phase. The solvent was allowed to move across the plate, after which the plate was taken out of the TLC chamber, air-dried, and then placed in the iodine chamber. Three distinct coloured spots were observed for the three eluted compounds. The RF value was calculated by measuring the distance travelled by the solute in relation to the distance travelled by the solvent, both in centimetres. The RF value was determined using the following formula: RF value = Distance travelled by the solute / Distance travelled by the solvent (Bele, A.A. and Khale, A., 2011).

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CHARACTERIZATION OF PIPERINE

Fractions obtained through column chromatography using benzene-EtOAc (3:1) were subjected to

concentration under reduced pressure, followed by recrystallization using a mixture of benzene and chloroform (7:1). This process resulted in the formation of pale yellow crystals identified as compound A2, likely piperine, yielding approximately 5 g (1.5%) with a melting point of 128~129oC (literature value 130oC). The structural elucidation of the purified crystalline compound A2 was achieved using spectroscopic techniques, including IR (Beć KB, et al 2020 & Lilo T, et al 2022), 1H NMR , and mass spectrometry (Rozanova S, et al 2021).

***In-vitro* Anti-Inflammatory Activity**

In vitro Egg Albumin Denaturation Method

Inhibition of Albumin Denaturation:

The response blend was made by combining 0.5 ml of Boswellic acid corrosive and a 0.45 ml fluid arrangement of 5% bovine egg whites. The pH of the blend, which was 6.3, was balanced with a bit of 0.1N HCl whereas keeping it at 37 ˚C for 20 minutes. After that, it was warmed to 57 ˚C for 30 minutes. Once cooled, the arrangement was exchanged to 96-well plates, and the absorbance was measured at 660 nm. Standard was utilized as Diclofenac sodium (1000µg/ml) and the control contain 0.05ml refined water (Dharmadeva S et al., 2016). The rate of restraint of egg whites denaturation was calculated by the taking after equation,

$$Percentage\ of\ Inhibition\ (\%) = \frac{Absorbance\ of\ Control - Absorbacne\ of\ Test\ Sample}{Absorbance\ of\ Control}\ x\ 100$$

Where, Control – Reaction mixture except drug, Test Sample – Reaction mixture containing the sample

RESULTS

Initial screening of the phytochemical constituents present in the ethanolic extract of pepper indicated the presence of phenols, flavonoids, and carbohydrates (Table 1and Fig 1). TLC Rf Value is 0.4 (Table 2 and Fig 2). The resulting mass analysis revealed a pair of major signals, but neither of them matched the predicted molecular ion for Piperine. Peak 1: 268.1905. This peak exhibited the highest intensity within the spectrum, setting aside any background noise, and emerged rapidly during elution (t_R = 0.215 min), indicative of a highly polar compound, a molecule of diminutive size, or possibly an ion linked to the matrix or a contaminant (Fig 3). Infrared (IR) spectroscopic examination of piperine, the data reveals the following characteristic peaks. The peak at 1579. 70 corresponds to the carbonyl group (C=O), the peak at 1435. 04 corresponds to the aromatic carbon-carbon bond (C=C Ar-H), the peak at 1012. 63 corresponds to the ether linkage (C-O-C), and the peak at 827.46 corresponds to the aromatic carbon-hydrogen bond (Ar-H) (Fig 4). 400 MHZ 1H NMR spectrum of compound A2 (piperine) (Fig 5) elucidated by MNR 6.8-7.1 , m ,8H, Pyridinyl H, 6.1 s,Ar-H, 6.5, d, IH, Ar-H, 7.2-7.3, m,2H, Ar-H, 1.4, s, CH, 1.6,s, CH, 3.4,s,CH, 3.6 s,CH . To assess anti-inflammatory activity in vitro, the albumin denaturation method was utilized. At extract 150 mg/ml, demonstrated a 54% inhibition and an IC_{50} value of 88.34 and at Compound 50 mg/ml, demonstrated a 59% inhibition and an IC_{50} value of 128.47 compared to the standard diclofenac sodium, which had an IC_{50} value of 121.29 mg/ml. When compared to diclofenac sodium, Piperine extract and Compound showed **good** anti-inflammatory effects (Table 5, 6 and Fig 6, 7).

Table 1: Phytochemical Studies – Piperine [- absence; + presence]

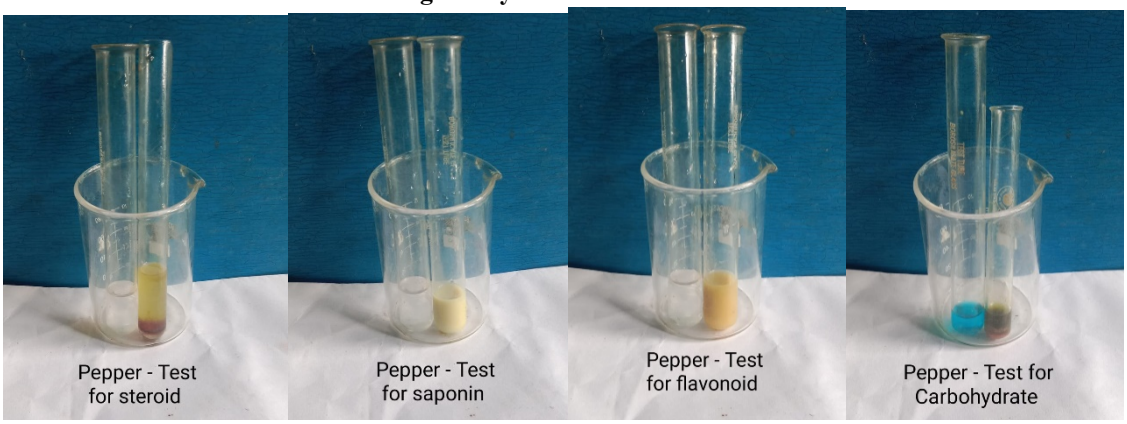
S.No	Phytochemical Test	Ethanol Extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Phenols	+
5.	Protein	+
6.	Saponin	+
7.	Carbohydrates	+
8.	Steroids	-

9.	Terpenoid	+

Table 2: Piperine- RF VALUE

	Piperine
Distance travelled by solvent	**5.9**
Distance travelled by solute	**2.5**
Rf Value	**0.4**

Fig 1: Phytochemical Studies



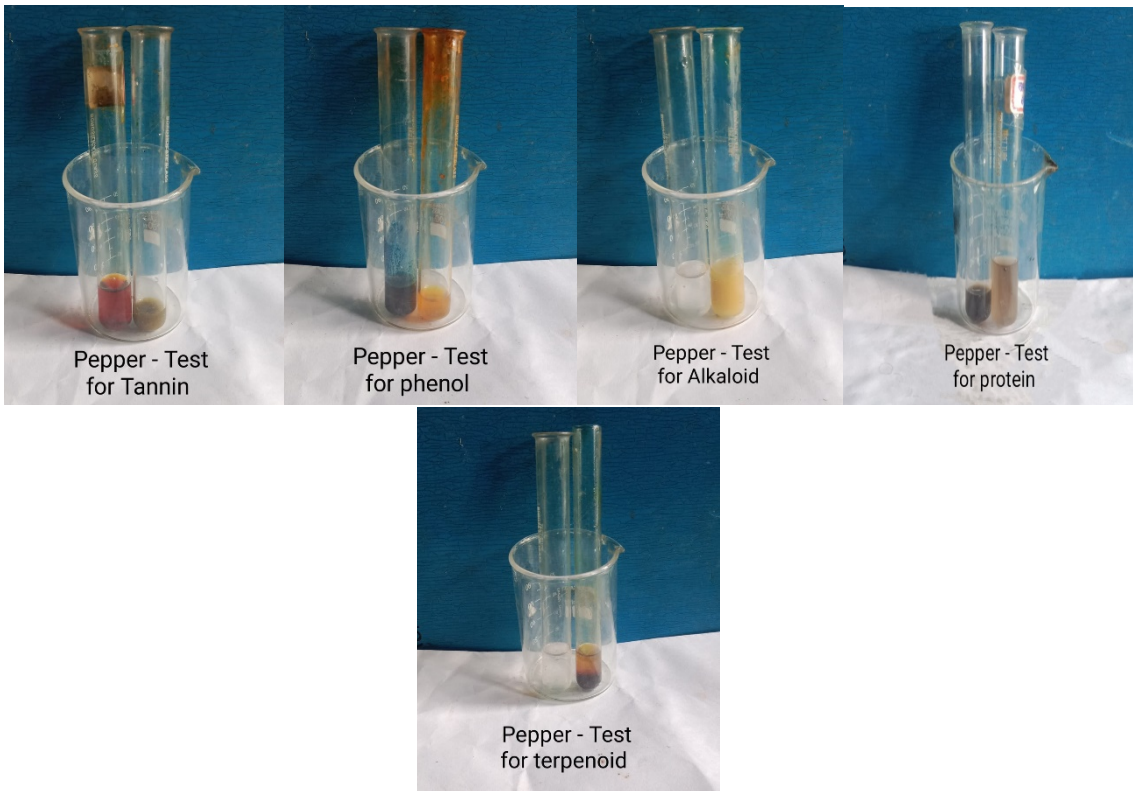
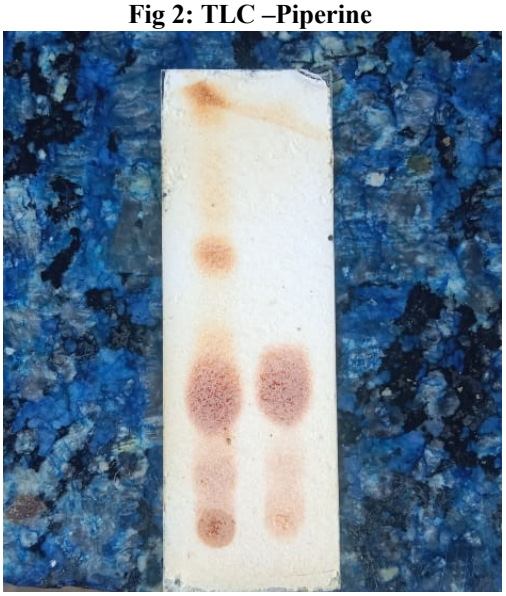


Fig 2: TLC –Piperine



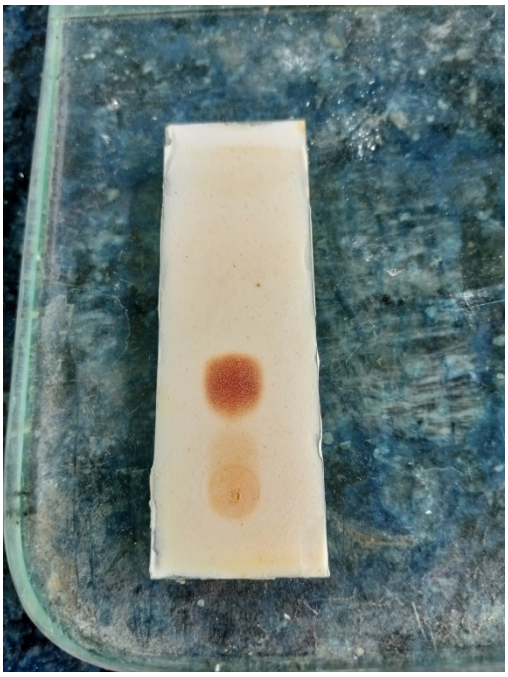


Fig 3: Piperine – Mass Spectrum

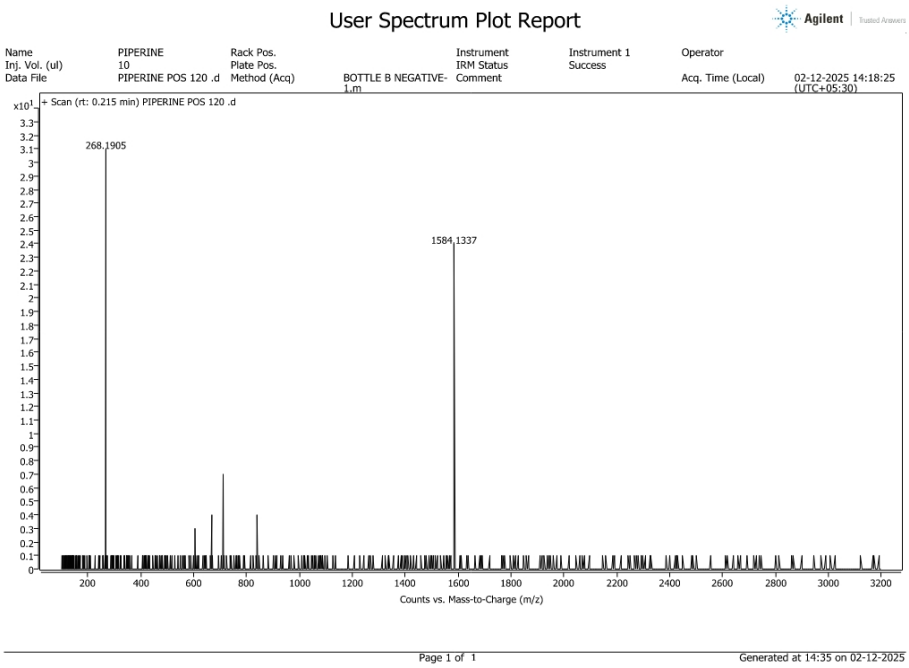
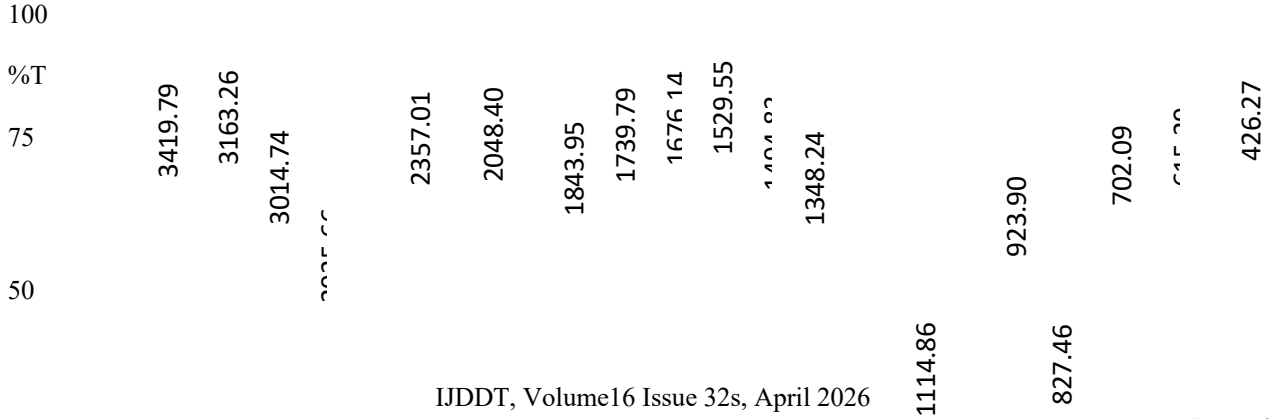


Fig 4: Piperine -IR Analysis



25 1579.70

Fig 5: 400 MHZ 1H NMR spectrum of compound A2 (piperine)

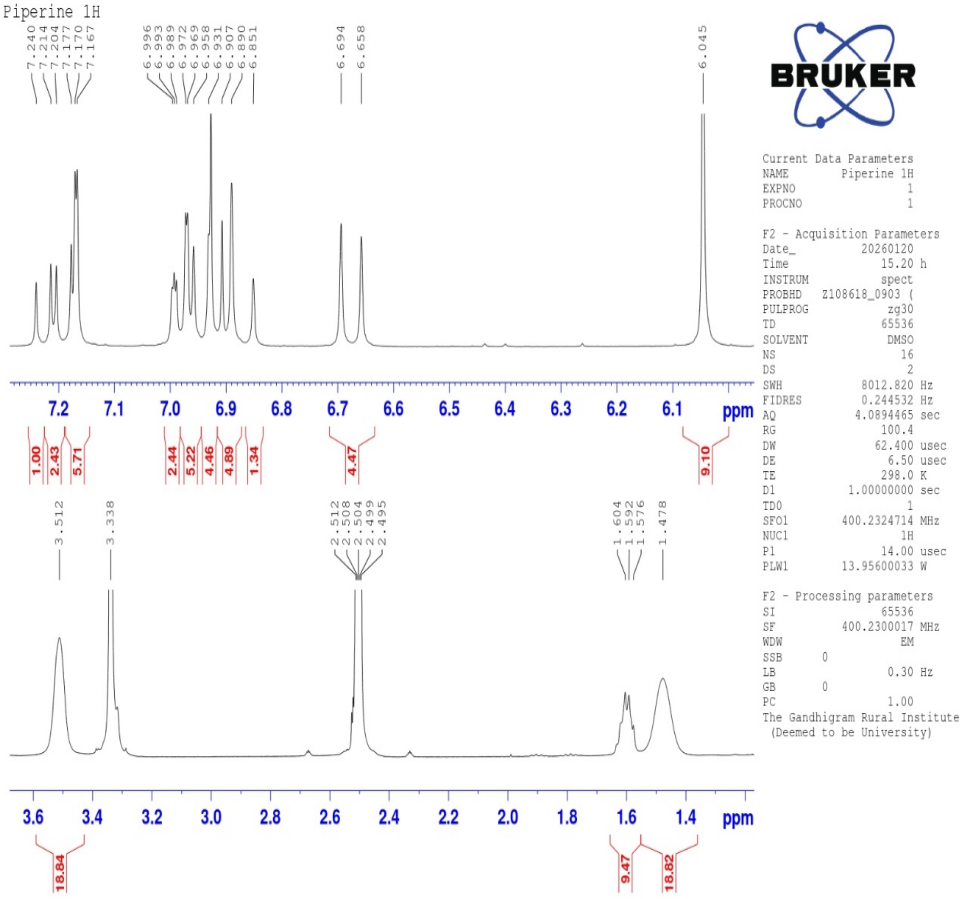


Table 5: Extract *In vitro* anti-inflammatory activity

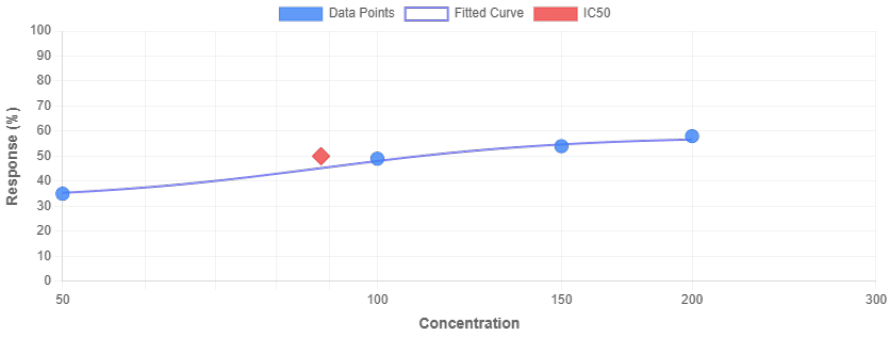
S.NO	Concentration(mg)	COD	SOD	%inhibition	Average	IC_{50} (mg/ml)
Extract						
1	50 mg	0.36	0.24	33%	34.6 ± 2.88	88.34 mg/ml
2		0.36	0.22	38%		
3		0.36	0.24	33%		
1	100 mg	0.36	0.18	50%	49 ± 1.73	
2		0.36	0.18	50%		
3		0.36	0.19	47%		
1	150 mg	0.36	0.16	55%		

2		0.36	0.16	55%	54 ± 1.73	
3		0.36	0.17	52%		
Compound						
1	50 mg	0.36	0.14	61%	59 ± 3.46	128.47 mg/ml
2		0.36	0.14	61%		
3		0.36	0.16	55%		
1	100 mg	0.36	0.14	61%	57 ± 4.58	
2		0.36	0.15	58%		
3		0.36	0.17	52%		
1	150 mg	0.36	0.19	47%	48 ± 1.73	
2		0.36	0.19	47%		
3		0.36	0.18	50%		
In vitro Standard Diclofenac sodium						
1	100 mg	0.36	0.06	83%	84 ± 1.73	
2		0.36	0.05	86%		
3		0.36	0.06	83%		

Table 6: Extract Anti-Inflammatory Activity Ic_{50} Value (mg/ml) Compared to Standard Diclofenac Sodium Ic_{50} Value (mg/ml)

S.NO	Concentration (mg)	Average (%)	IC_{50} (mg/ml)
Extract			
1	50 mg	35%	88.34 mg/ml
2	100 mg	49%	
3	150 mg	54%	
Compound			
1	50 mg	59%	128.47 mg/ml
2	100 mg	57%	
3	150 mg	48%	
Standard Diclofenac sodium			
1	50 mg	91%	**121.29 mg/ml**
2	100 mg	93%	
3	150 mg	85%	
4	200 mg	89%	
5	250 mg	88%	

Fig 6: Anti-Inflammatory Activity Ic_{50} Value (mg/ml)
Extract



Compound

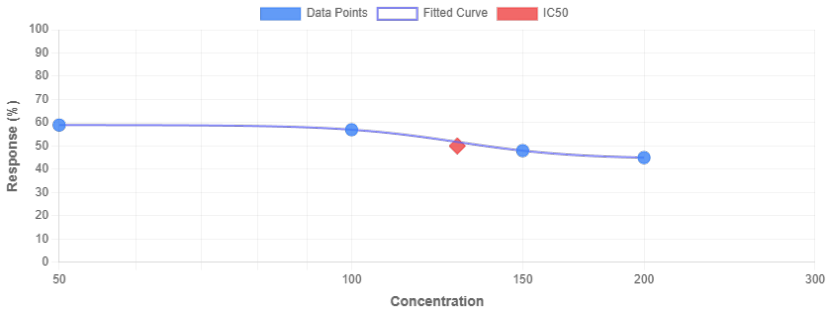
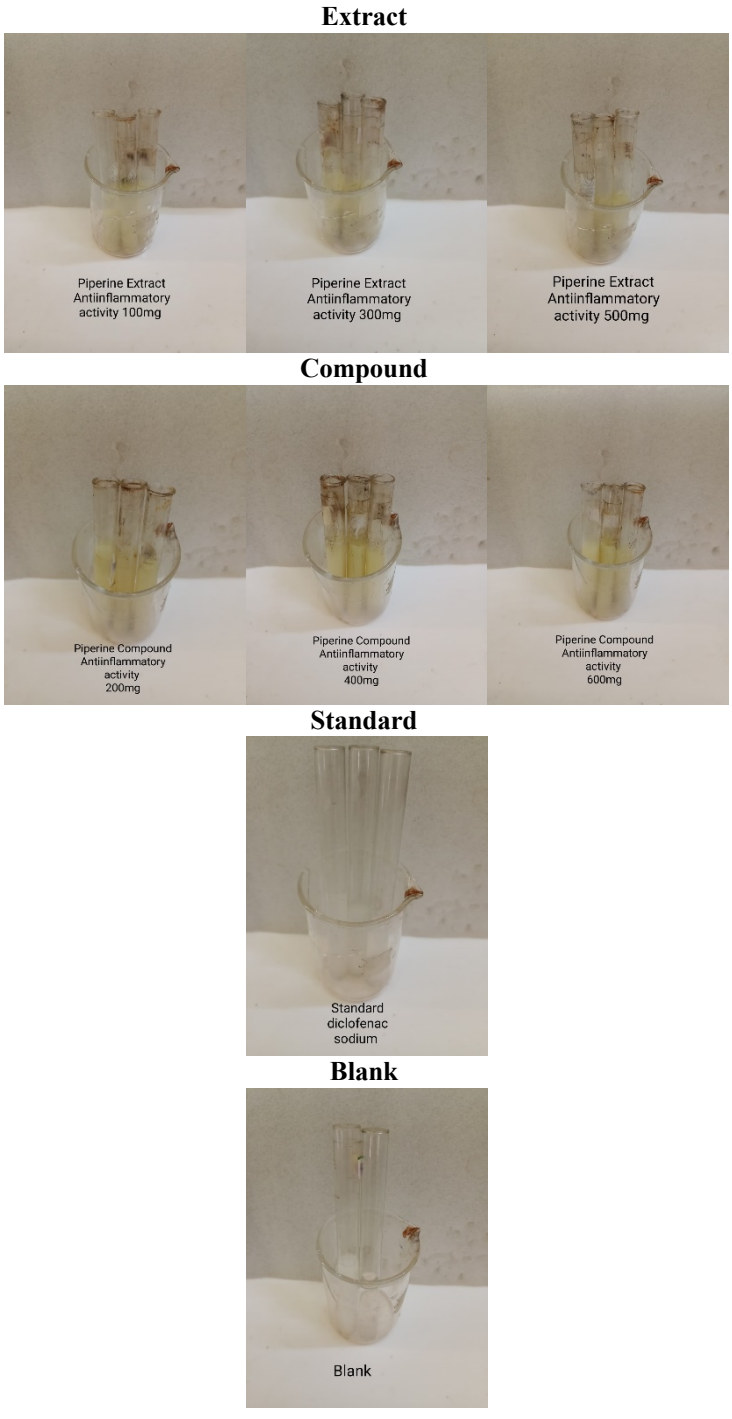


Fig 7: *In vitro* anti-inflammatory activity



DISCUSSION

The egg albumin denaturation test is carried out to ascertain whether a drug or substance has the ability to halt or reduce the denaturation of egg albumin, which, as demonstrated in a prior study (Dharmadeva S et al., 2018), indicates its anti-inflammatory properties. Piperine (1-peperoyl piperidine) was extracted from Piper nigrum Linn to assess its anti-inflammatory properties in rats. Various experimental models, both acute and chronic, were utilized, including carrageenan-induced paw edema in rats, cotton pellet granuloma, and croton oil-induced granuloma pouch. Concurrently, biochemical measurements were taken to clarify the mechanisms behind its actions. Piperine exhibited a significant impact on initial acute inflammatory responses as well as chronic granulomatous developments. It appeared to operate partially through the stimulation of the pituitary-adrenal axis. However, exudative changes in both acute and chronic models were found to be insignificant (Mujumdar, A.M., et al 1990). The anti-inflammatory effects of piperine were tested using carrageenan-induced paw edema in rats. Sprague Dawley Rats were categorized into control groups that received saline orally, while the piperine groups were administered doses of 2.5; 5; and 10 mg/kg of piperine (also orally). Subsequently, 1% carrageenan was delivered via the intraplantar route 30 minutes following the piperine or saline administration. Measurement of paw volume and prostaglandin production was conducted after the carrageenan injection. The piperine doses of 2.5; 5; and 10 mg/kg displayed 5.4; 43.8; and 54.8% reductions in paw edema, respectively, after a three-hour period. The rise in prostaglandin (PGE2) levels following carrageenan injection was notably reduced by the piperine administration at 5 and 10 mg/kg, though not by the 2.5 mg/kg dose. These findings imply that the anti-inflammatory effects of piperine may be due to the suppression of prostaglandin release (Sudjarwo, S. A., 2005). Piperine and aspirin demonstrated significantly reduced paw edema when compared to the negative control group. In a comparison between the aspirin group and the piperine group, there was no observable difference at the 2-hour mark; however, at 3 hours, the piperine group exhibited notably higher paw edema than the aspirin group. The percentage inhibition observed at 3 hours was 61.57% for the aspirin group and 55.81% for the piperine group compared with the control group (Dhargawe, N., et al 2021).

CONCLUSION

The examination of phytochemicals and the analysis of structure (IR, NMR, and TLC) in the extract of piperine. Laboratory tests revealed that both the extract and isolated piperine exhibit good anti-inflammatory effects, considerably reducing albumin denaturation. This research confirms piperine as a powerful natural option for therapeutic applications related to inflammation.

REFERENCE

1. Chopra B, Dhingra AK, Kapoor RP, Prasad DN. Piperine and its various physicochemical and biological aspects: a review. Open Chem J 2016;3. DOI: 10.2174/1874842201603010075.
2. Sozzi GO, Peter K V, Babu KN, Divakaran M. Capers and caperberries. Handb. herbs spices, Elsevier; 2012, p. 193–224.
3. Pruthi JS. Quality assurance in spices and spice products, modern methods of analysis 1999. ISBN: 9788170238966
4. Ravindran P. 2003. Black pepper: Piper nigrum. Boca Raton, Fla.: CRC Press. ISBN 9789057024535
5. Hirasa K, Takemasa M. 1998. Spice science and technology. Boca Raton, Fla.: CRC Press. ISBN 9780824701444
6. Kozukue N, Park M-S, Choi S-H, Lee S-U, Ohnishi-Kameyama M, Levin CE, et al. Kinetics of light-induced cis− trans isomerization of four piperines and their levels in ground black peppers as determined by HPLC and LC/MS. J Agric Food Chem 2007;55:7131–9. DOI: 10.1021/jf070831p.
7. Zachariah TJ, Parthasarathy VA. 2008. Black pepper. Chem spices. 196:21. ISBN-13: 9781 845934057
8. Pruthi JS. Major spices of India: crop management and post-harvest technology. Major Spices Crop Manag Post-Harvest 2004.
9. Hlavačková L, Janegová A, Uličná O, Janega P, Černá A, Babál P. Spice up the hypertension diet-curcumin and piperine prevent remodeling of aorta in experimental L-NAME induced hypertension. Nutr Metab (Lond) 2011;8:72. DOI: 10.1186/1743-7075-8-72.
10. Li S, Lei Y, Jia Y, Li N, Wink M, Ma Y. Piperine, a piperidine alkaloid from Piper nigrum re-sensitizes P-gp, MRP1 and BCRP dependent multidrug resistant cancer cells. Phytomedicine 2011;19:83–7. DOI: 10.1016/j.phymed.2011.06.031
11. Zarai Z, Boujelbene E, Salem N Ben, Gargouri Y, Sayari A. Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from Piper nigrum. LWT-Food Sci Technol 2013;50:634–41. DOI: 10.1016/j.lwt.2012.07.036
12. Miyakado M, Nakayama I, Yoshioka H, Nakatani N. The Piperaceae amides I: Structure of pipercide, a new insecticidal amide from Piper nigrum L. Agric Biol Chem 1979;43:1609–11. DOI: 10.1080/00021369.1979.10863675
13. Lee SA, Hong SS, Han XH, Hwang JS, Oh GJ, Lee KS, et al. Piperine from the fruits of Piper longum with inhibitory effect on monoamine oxidase and antidepressant-like activity. Chem Pharm Bull 2005;53:832–5 DOI: 10.1248/cpb.53.832
14. Stahl E, Schild W (1981) Pharmazeutische biologie—drogenan alyse II: inhaltsstoffe und isolierungen. Gustav Fischer Verlag, New York, pp 410–411
15. Farnsworth NR. Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences 1966; 55(3): 225-76.

16. Haseen, A., Prasanth, M.L. and Jagajith, A.A., 2024. Comprehensive study on phytochemical analysis of medicinal plants. *International Journal of Pharmacognosy*, *11*(3), pp.53-64.
17. Bele, A.A. and Khale, A., 2011. An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research*, *2*(2), p.256.
18. Beć KB, Grabska J, Huck CW. Near-Infrared Spectroscopy in Bio-Applications. Molecules. 2020 Jun 26;25(12):2948. doi: 10.3390/molecules25122948. PMID: 32604876; PMCID: PMC7357077.
19. Lilo T, Morais CLM, Shenton C, Ray A, Gurusinghe N. Revising Fourier-transform infrared (FT-IR) and Raman spectroscopy towards brain cancer detection. Photodiagnosis Photodyn Ther. 2022 Jun;38:102785. doi: 10.1016/j.pdpdt.2022.102785. Epub 2022 Feb 26. PMID: 35231616.
20. Rozanova S, Barkovits K, Nikolov M, Schmidt C, Urlaub H, Marcus K. Quantitative Mass Spectrometry-Based Proteomics: An Overview. Methods Mol Biol. 2021;2228:85-116. doi: 10.1007/978-1-0716-1024-4_8. PMID: 33950486.
21. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. *In vitro* anti-inflammatory activity of *Ficusracemosa* L. bark using albumin denaturation method. Ayu. 2018 Oct-Dec;39(4):239-242. doi: 10.4103/ayu.AYU_27_18. PMID: 31367147; PMCID: PMC6639822.
22. Mujumdar, A.M., Dhuley, J.N., Deshmukh, V.K., Raman, P.H. and Naik, S.R., 1990. Anti-inflammatory activity of piperine. *Japanese Journal of Medical Science and Biology*, *43*(3), pp.95-100.
23. Sudjarwo, S.A., 2005. The potency of piperine as antiinflammatory and analgesic in rats and mice. *Folia Medica Indonesiana*, *41*(3), pp.190-194.
24. Dhargawe, N., Mahakalkar, S., Mohod, B. and Raj, J.P., 2021. Evaluation of analgesic, anti-inflammatory, and antipyretic activity of piperine: an experimental study. *Pharmacognosy Research*, *12*(2).