A Review on the Therapeutic Potential of Black Pepper (*Piper nigrum L.*)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "A Review on the Therapeutic Potential of Black Pepper (*Piper nigrum L.*)" submitted by Mubasshira Maleka Meem (ID 19146053) of Spring, 2019 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study does not involve any kind of animal trial or human trial.

Abstract

Spices have been a crucial component of human diets and trade for thousands of years. Due to their medicinal potential for treating many illnesses, their bioactive components are extremely important. They frequently fall under the category of functional foods because they not only give essential nourishment but also physiological advantages or prevent chronic illness. Due to its anti-oxidant, anti-microbial, and gastro-protective components, black pepper (*Piper Nigrum L.*) is a significant healthy food. Piperine, the active component in black pepper, is part of a complex phytochemistry that also includes volatile oil, oleoresins, and alkaloids. More recently, animal modelling and research on cell cultures anticipated that black pepper effectively treats a variety of diseases. The current view of this study is to highlight the different therapeutic importance of *Piper nigrum* as well as its future prospects and drawbacks which can be solved using various modern approaches.

Keywords: *Piper nigrum*; Black Pepper; Therapeutic potential; Piperine

Dedication

I would to dedicate this thesis to my parents, who did everything possible to ensure I would have the opportunity of an education. Their immensible encouragement, efforts and struggle have helped me to unlock the mysteries of our world, and beyond.

Acknowledgement

Firstly, I would like to thank Almighty Allah, for letting me through all difficulties. Without His blessings, I could not have made it this far.

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List of Acronyms

ROS	Reactive Oxygen Species
HUVEC	Human Umbilical Vein Endothelial Cell
PARP	Poly ADP-Ribose Polymerase
CML	Chronic Myelogenous Leukaemia
PCNA	Proliferating Cell Nuclear Antigen
HER2	Human Epidermal Growth Factor Receptors
FAS	Fatty Acid Synthase
siRNA	small interfering ribonucleic acid
DAPK2	death-associated protein kinase 2
5-FU	5-Fluorouracil
LPS	Lipopolysaccharide
GABA	Gamma-aminobutyric acid
FFA	Free Fatty Acid
TLR	Toll Receptor
PDE	Phosphodiesterase
MES	Maximal electroshock seizure
PTZ	Pentylenetetrazol
LPL	Lipoprotein lipase
LCAT	Lecithin cholesterol acyltransferase
NO	Nitric Oxide
ACE	Angiotensin Converting Enzyme
NADPH	Nicotinamide adenine dinucleotide phosphate

AHH	Aryl hydrocarbon hydroxylase
UDP-GDH	Uridine diphosphate glucose dehydrogenase
UDPGT	Uridine Diphosphate-glucuronyl-transferase
MAO	Monoamine oxidase inhibitors
UGT	UDP-glucuronyl transferase
UDPGA	UDP-glucuronic acid
LOX	Lipoxygenase
HME	Hot Melt Extrusion
GMP	Good Manufacturing Practice

Chapter 1

Introduction

1.1 Background

Due to their wide range of pharmacological potentials and fewer adverse effects on biological systems, medicinal plants are particularly well-liked in several traditional systems of medicine. Among several herbs, *Piper nigrum L.* is a popular spice known as "The King of spices." It contains the potent alkaloid "piperine," which has a wide range of pharmacological effects. By blocking a number of metabolizing enzymes, piperine boosts the bioavailability of several medications. Antimicrobial, antioxidant, antitumor, anti-asthmatic, analgesic, anti-inflammatory, anti-diarrheal, antidepressant, immunomodulatory, anticonvulsant, hepato-protective, digestion-improving, and other pharmacological activities are just a few of the pharmacological properties of *Piper nigrum L.* and its active ingredient, "Piperine." The objective of the current review is to present an updated overview of the literature on significant progress in pharmacological activity research and the potential applications of *Piper nigrum L*. A review of the *Piper nigrum*'s therapeutic potential has been compiled by gathering the most comprehensive data from reliable sources online.

1.2 Objectives of the Study

Piper nigrum is called the ‘King of Spices’ which has several traditional uses starting from aiding as a flavouring agent in food to providing many health benefits. The goal of the study is to provide a comprehensive review of the therapeutic potential of *Piper nigrum* and highlight the prospects of black pepper research.

1.3 Rationale of the Study

Plants have always been an important source of chemicals serving several medicinal purposes. *Piper nigrum* is one of the most commonly used spices in almost all continents, especially in Asia. The fruits of this flowering vine of the Piperaceae family can be treated in different ways to obtain black pepper, green pepper, red pepper and white pepper. Due to the broad range of health

benefits provided by several parts of *Piper nigrum* including the flower, leaf, stem, seed and fruit, this plant has been widely studied to identify the bioactive phytochemicals responsible for the therapeutic effects followed by the investigation of different biological properties, which remained initially undiscovered, in the process. Medicinal plants have various pharmacological potentials and fewer adverse effects which makes it more essential to explore their therapeutic potential along with the mechanisms by which the chemical constituents exhibited their work. In this review paper, we could enrich our knowledge about the beneficial biological aspects of *Piper nigrum* and its coordinative effect with various medications. Additionally, we will learn how different plant parts act individually to achieve various health benefits. This study will also help us to identify the potential compounds in *Piper nigrum* with different pharmacological activities.

Chapter 2

Methodology

The information for this study was collected from Scopus, PubMed, Science Direct, and ResearchGate, as well as from other authenticated and renowned websites. Firstly, an outline was designed for executing the review in a sorted manner. The keywords for searching relevant information included "*Piper nigrum*", "Black Pepper", "Therapeutic Potential of Black Pepper", "Biological role of Black Pepper", "Future prospects of black pepper", "Role of piperine in the human body", etc. All the information included in this paper was cited at the end of this article. This study aims to briefly highlight the therapeutic potential of *Piper nigrum* and its future prospects.

Chapter 3

Piper nigrum

3.1 Taxonomic Classification

Kingdom: Plantae

Class: Equisetopsida

Subclass: Magnoliidae

Superorder: Magnolianae

Order: Piperales

Family: Piperaceae

Genus: Piper

Species: nigrum (Singh, 2017)

3.2 Geographic Distribution

Black pepper is a popular spice worldwide and most of its centre of origin is South West India, precisely the Western Ghats region of South India. It was the first oriental spice to be introduced into the Western world. Afterwards, during the middle age, it earned popularity in Europe. Initially, black pepper used to be cultivated only in the western coastal region of India whereas, now its cultivation has emerged in most tropical countries. Nowadays, tropical zones of the Asia-Pacific region, mainly India, Indonesia, Malaysia, Sri Lanka, Thailand, Cambodia and Vietnam grow pepper. Other than that, black pepper is widely distributed in a total of about 26 countries which includes Brazil and Mexico. It has become a major agricultural exporting commodity as well as it is also the most widely traded spice (Ravindran & Kallupurackal, 2012)**.**

3.3 Botanical Characteristics

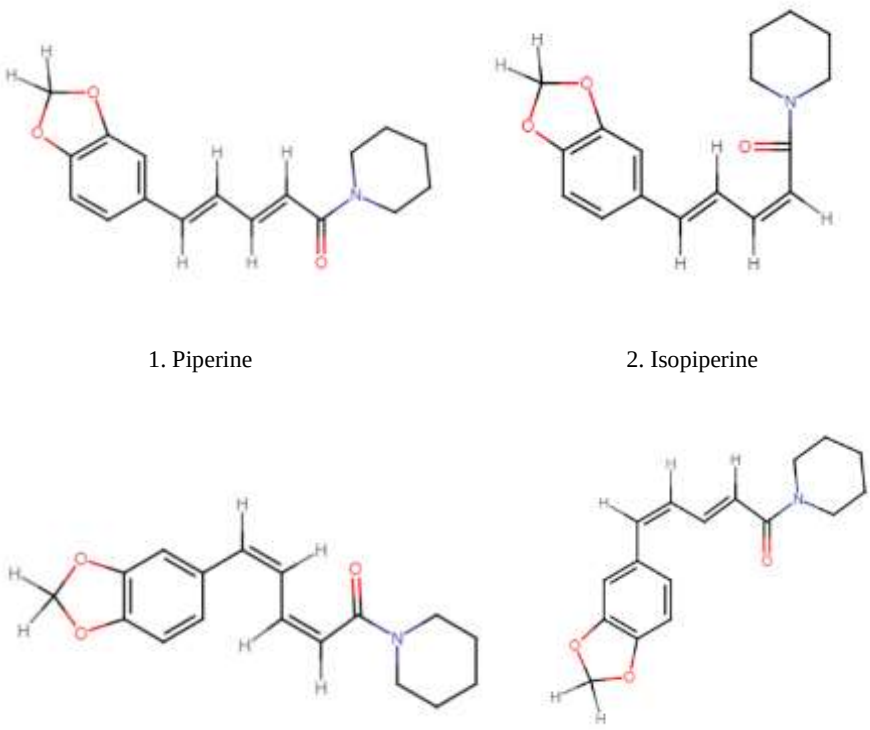
Black pepper is the product of the mature fruits of *Piper nigrum* which is a perennial woody climbing vine. They can easily grow staying under the shade of supporting trees, trellises or a pole with a maximum height of 13 feet or 4 metres. The putative parents for *Piper nigrum* are

three species that include *P. galeatum*, *P. trichostchyon* and *P. wightii*. Their study also described that the black pepper plant has around 10 to 12 adventitious roots evolving from the base of its mature stem. Moreover, the plant has a dimorphic branching and orthotropic shot which helps it to climb over the supporting trees (Ashokkumar et al., 2021). The structure of the leaf of *P. nigrum* carries pendant spikes (catkins) on its opposite part whose length extends up to 7 to 15 cm. Each stem contains 20 to 30 spikes of fruits, also known as peppercorns which are isolated and sun-dried to make black pepper (Damanhouri, 2014). This process initiates with soaking the fruits in boiling water for 10 minutes. Due to it, the exterior of the fruits gets disinfected and the fermentation process starts which finally gives them a black colour (Milenković & Stanojević, 2021). The fruit often called a berry is small and usually spherical in shape and is a single-seeded drupe. Moreover, it has a fleshy pericarp and hard endocarp as shown in Figure 1 (Ravindran & Kallupurackal, 2012)**.** Piperine and starch contents increase during the maturation of fruits while the volatile oils and non-volatile resins decrease. Hence, harvesting the fruit should be done carefully to ensure a uniform and high-quality final product (Dahmani et al., 2019).

Figure 1: Different botanical features of Black Pepper (Ashokkumar et al., 2021)

3.4 Phytochemistry

Black pepper is enriched with nutrients, vitamins and minerals. 100-gram seeds of black pepper contain 66.5 g of carbohydrates, 10 g of protein and 10.2 g of fat. Besides, it contains a significant mineral content for instance 400 mg calcium, 235.8-249.8 mg magnesium, 1200 mg potassium, 160 mg phosphorus and lower concentrations of iron, sodium and zinc. Moreover, there is a high number of vitamins like Vitamin B1, B2, B3 and C can also be found (Ashokkumar et al., 2021). Black pepper's quality is conserved by two components which are volatile oil and piperine. Piperine is responsible for pungency while volatile oil is responsible for the aroma and flavour of black pepper (Ravindran & Kallupurackal, 2012). Piperine and other major alkaloids like piperidine, piperetine and chavicine are the reason for the pungent attribute of black pepper. He also described that piperine exerts various health-provoking potentials like facilitating digestion, providing liver protection, improving the drug's bioavailability and aiding in the bioabsorption of micronutrients, vitamins and trace elements. Black pepper is enriched with potent phenolic acids and antioxidants. Phenolic acids refer to the mixture of glycosides of phenolic acid and flavanol glycosides. Other essential constituents include oleoresins, terpenes, alkamides, lignans, steroids and flavones. As previously mentioned, the essential oil is responsible for aroma while oleoresin contributes to the overall taste of black pepper (Butt et al., 2013)**.** Black pepper has more than 250 volatiles, with the principal constituents including α-phellandrene, β-caryophyllene, α-pinene, β-pinene, cis-β-ocimene, germacrene D and limonene (Dahmani et al., 2019). In addition, four isomeric forms are present in black pepper (Figure 2); Piperine (trans-trans isomer), Isopiperine (cis-trans isomer), Chavicine (cis-cis isomer) and Isochavicine (trans-cis isomer) (Milenković & Stanojević, 2021). Compared to black pepper, white pepper, and black pepper methanol extract, the pericarp had a higher quantity of total phenols, but it also had a lower piperine content (Lee, Chae, et al., 2020). After the black pepper's outer peel was removed, the amount of piperine increased while the number of total flavonoids, phenols and minerals decreased. In terms of bioactivity and pungency, utilizing pepper without the outer skin is therefore preferable as it has a more delicate flavour and stronger antioxidant capacity (Lee, Kim, et al., 2020).



1. Piperine

2. Isopiperine

3. Chavicine

4. Isochavicine

Figure 2: Four isomeric forms present in Black Pepper (Gorgani et al., 2017)

Chapter 4

Therapeutic Potential of *Piper nigrum*

4.1 Antimicrobial Activity

Piperine has antibacterial capabilities and can significantly slow down the development of *Salmonella typhi*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. With inhibitory concentrations of 125, 250, and 500 ppm, respectively, several black pepper extracts demonstrated effective growth inhibition of bacteria like Staphylococcus, Bacillus, and Streptococcus (Butt et al., 2013)**.** Black pepper has antimicrobial properties against a variety of bacteria, including *E. coli*, *S. typhimurium*, *P. aeruginosa*, and *B. subtilis*, and also against fungi such as *F. oxysporum*, *A. niger*, and *Candida spp***.** The development of foodborne pathogens such as *E. coli*, *B. cereus*, *S. aureus*, and *S. typhimurium* was found to be inhibited by two substances from black pepper, 3,4-dihydroxy-phenyl ethanol glucoside and 3,4-dihydroxy-6-(N-ethyl-amino) benzamide**.** Additionally, Among the several solvent extracts (hexane, dichloromethane, ethanol, and aqueous), the black pepper fruit's dichloromethane extracts showed the greatest effectiveness against the bacteria *S. aureus*, *E. coli*, *S. typhi*, and *B. subtilis*. Piperine showed lower antibacterial activity compared to piperic acid against many Gram-positive and Gram-negative bacteria. Besides, black pepper oil did not exhibit an inhibitory effect against Gram-negative organisms like *E. coli* and *Salmonella sp.* in Hikal's investigation**.** The black pepper fruit's crude extracts demonstrated strong anticandidal action and might be utilized to treat oral and vaginal candidiasis brought on by distinct species of Candida (Milenković & Stanojević, 2021)**.**

Piperine has been discovered to inhibit methyltransferase of Dengue infection and VP35 interferon inhibitory space of Ebola infection in comparison to commercial antiviral Ribavirin. Black pepper consists of the bioactive compounds piperdardine and piperanine which are potent inhibitors of COVID-19**.** The chloroform extract of *Piper nigrum* has more alkaloids, which gives it a higher antiviral activity. In contrast to multidrug-safe gram-positive bacteria, *Staphylococcus aureus* (DIZ-18 mm), rough piperine extracted from *Piper nigrum* displayed the

strongest antibacterial and antifungal activity toward *Fusariumoxysporum* (DIZ-14mm) (Jogdand & Singh, 2021)**.**

4.2 Antioxidant Activity

Black pepper is a significant source of natural antioxidants. The primary function of antioxidants is to shield cells from free radicals, which have been linked to heart disease, cancer, and other illnesses. Ascorbic acid, b-carotene, camphene, eugenol, carvacrol, g-terpinene, lauric acid, myrcene, linalyl acetate, methyl-eugenol, myristic acid, myristicin, palmitic acid, piperine, terpinen-4-ol, and ubiquinone were the antioxidant active compounds obtained from black pepper (Dahmani et al., 2019). Additionally, black pepper holds an important place in disease prevention measures related to reactive oxygen species (ROS) and related species due to the synergistic effects of piperine with some other antioxidants like curcumin. Supplementing with piperine or black pepper reduced oxidative damage and altered how the body's enzyme systems worked (Butt et al., 2013)**.** These antioxidant mechanisms can be explained by the potent hydrogen donating, metal chelating, free radical, hydrogen peroxide, and superoxide scavenging properties of black pepper (Gülçin, 2005).

Numerous independent researchers have used experiments to show that piperine has many physiological effects. It has restricted oxidative damage by preventing or quenching free radicals, reducing lipid peroxidation, and having a positive impact on cellular thiols, antioxidant molecules, and antioxidant enzymes under various oxidative stress scenarios (Dahmani et al., 2019)**.** Piperine or *Piper nigrum* was also demonstrated to reduce lipid peroxidation *in vivo*. According to reports, *Piper nigrum* has antioxidant properties for containing flavonoids and phenolic compounds. *Piper nigrum* was discovered to reduce lung carcinogenesis in animal tests by decreasing human lipoxygenase, lipid peroxidation, halting hydroxyl as well as superoxide free radicals (Damanhouri, 2014). The modification in lipid peroxidation and the stimulation of antioxidant enzymes reduce the lung metastatic spread in the B16F-10 melanoma cells (Shityakov et al., 2019)**.**

Additionally, piperine lowers the quantity of thiobarbituric acid reactive chemicals by balancing the quantities of catalase, glutathione, glutathione peroxidase, glutathione-S-transferase, and superoxide dismutase. Superoxide dismutase, glutathione peroxidase, and glutathione-s-

transferase levels expanded in the liver of the treated groups, suggesting that the treatment may have some protective effects against the cellular damage caused by free radicals in the body (Jeena et al., 2014).

4.3. Anti-tumour and Anti-cancer Activity

Numerous researchers have investigated the anticancer properties of black pepper, particularly piperine, against various cancer cell lines. On the three colorectal cancer cell lines HT-29, HCT-116, and HCT-15, it was discovered that the ethanolic extract of the black pepper fruit had an anticancer impact. Black pepper root extracts in petroleum ether and chloroform demonstrated cytotoxicity when applied to human myeloid leukaemia HL-60 cells. The MCF-7 and MDA-MB-231 breast cancer cell lines were more sensitive to the methanolic extract of black pepper fruit than to the dichloromethane extract. Black pepper fruit ingredient piperlonguminine demonstrated cytotoxic properties against breast and colorectal cancer cells. Besides, piperine can restrict the G1 or S phase of the cell cycle, which prevents human umbilical vein endothelial cells (HUVEC) from proliferating and migrating. In animal models of angiogenesis, piperine can prevent endothelial cells from forming tubules and protein kinase B from becoming phosphorylated (Milenković & Stanojević, 2021). Furthermore, it was discovered that piperine was effective against LNCaP, 22RV1, PC-3, and DU-145 prostate cancer cell lines, which are both androgenic and independent. Piperine-induced apoptosis by activating PARP-1 (Poly [ADP-ribose] polymerase 1) and caspase-3 (Jogdand & Singh, 2021)**.** Additionally, Ser 473 and Thr 308 residues of protein kinase B's Akt are not phosphorylated by piperine. Apparently, phosphorylation of these is a crucial regulator of angiogenesis and endothelial cell function. Since angiogenesis is vital for the development of tumours, piperine might be utilized as an angiogenesis inhibitor in cancer treatment (Damanhouri, 2014).

Black pepper and its primary metabolite, piperine, are strong inhibitors of leukemic cells' ability to proliferate. To induce apoptosis in human chronic myelogenous leukaemia (CML) cells, these phytochemicals upregulate genes implicated in the intrinsic route, such as caspase-9, -3, and Bax, and then suppress the expression of proliferating cell nuclear antigen (PCNA) (as mentioned in Table 1. Both piperine and black pepper have a greater ability to cause cell death when time and dosage are applied. The findings of the study support the notion that including black pepper in a regular diet may help prevent the onset of CML (Banerjee et al., 2021).

Additionally, piperine and black pepper essential oil have anti-cancer properties. Prostate cancer cells that were both androgen-dependent and androgen-independent experienced a considerable reduction in tumour growth. Similarly, piperine decreased lung cancer by enhancing antioxidant protection enzymes and by lowering lipid peroxidation (Ashokkumar et al., 2021).

Following its oral administration to lower the extent of some types of gastrointestinal malignancies, piperine has shown anti-tumour effects. By modifying lipid peroxidation, which spreads free radical reactions and causes cellular damage, a black pepper alcohol extract with piperine is helpful against lung cancer. When used along with the FDA-approved anti-cancer drug docetaxel to treat castrate-resistant prostate cancer, piperine has some anti-cancer properties. The rate at which this medicine is metabolized in the liver is decreased by piperine via limiting the hepatic CYP3A4 enzyme's activity. The use of piperine in a nutritional supplement augments the immunosuppressive effects of docetaxel in xenograft animal models without producing side effects (Shityakov et al., 2019).

The most aggressive tumours in breast cancer are those that overexpress the human epidermal growth factor receptors (HER2). Numerous traits of malignant cells, such as unchecked proliferation, resistance to apoptosis, and enhanced motility, are conferred by HER2. HER2 expression by inhibiting HER2 gene transcription in HER2-overexpressing breast cancer cells since it efficiently suppressed HER2 promoter activity and mRNA level, resulting in HER2 protein level reduction. HER2 overexpression is a mediator of the stimulation of fatty acid synthase (FAS) in HER2-over-expressing breast cancer cells. Treatment with FAS small interfering ribonucleic acid (siRNA) results in malonyl-CoA accumulation, which inhibits carnitine palmitoyl transferase I. It increases ceramide levels, as well as the expression of the proapoptotic genes BNIP3, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), and death-associated protein kinase 2 (DAPK2). For this reason, FAS is thought to be an excellent target for cancer treatment. Piperine demonstrated a potent suppression of FAS gene expression in the study, and this inhibition helped to cause apoptosis in breast cancer cells that overexpress the HER2 gene. Results also revealed that piperine significantly reduced ERK1/2 activation, which in turn hindered SREBP-1 gene expression and decreased levels of mature SREBP-1. Piperine thereby reduced FAS expression by preventing SREBP-1 activation through the suppression of ERK1/2 signalling (Do et al., 2013).

Furthermore, MCF-7 cells were cytotoxic to *Piper nigrum* extract, which had an antiproliferative impact. Through oxidative cleavage and intercalation, the extract was able to attack DNA. By halting the cell cycle and inducing apoptosis, it prevented the formation of Ehrlich ascites cancer in vivo. Additionally, in vivo, the antitumor effect was linked to ROS overload in tumour cells that were kept in a pro-oxidative state, which was indicated by noticeably increased activity of the antioxidant enzymes CAT, SOD, and GR as well as a significant increase in the contents of lipid peroxidation and protein carbonylation (de Souza Grinevicius et al., 2016).

The above-mentioned functions along with many other functions are described in Table 1.

Table 1: Effects of piperine on different types of cancer (Zadorozhna et al., 2019)

Tumour entity	**Functions of piperine**
Breast Cancer	The arrest of the cell cycle at different checkpoints in relation to cancer cell lines; unbalance of ROS homeostasis and induction of apoptosis
Prostate Cancer	Interruption of the cell cycle at G0/G1 checkpoint through the inhibitory activity of CDK; down-regulation of p21 and p27; induction of apoptosis by means of caspase-3 and PARP cleavage
Osteosarcoma	Blockage of the cell cycle at the G2/M phase by down-regulating cyclin B1 and by increasing the phosphorylation level CDK1 and Chk2
Melanoma	Arrest of cell cycle at G1 phase through downregulation of cyclin D1, activation of CDK (p21/WAF1) and unbalance of ROS homeostasis
Colon Carcinoma	Downregulation of D1 and D2 cyclins and their partner CKD4 and CDK6 with the consequent arrest of the cell cycle at G1 phase; mTORC1 inhibitory activity promoting cancer cell death

Fibrosarcoma	Inhibition of PKCα and ERK1/2 phosphorylation and reduction of NF- κB and AP-1 nuclear translocation, leading to downregulation of MMP-9 expression
Ovarian Cancer	Activation of the intrinsic pathway of apoptosis after the release of mitochondrial cytochrome c to cytosol, the activation of caspase-3 and -9, the PARP cleavage and the inactivation of p38/MAPK and JNK
Lung Cancer	Reduction of oxidative stress mediated by mitochondrial activities and enhancement of both enzymatic and non-enzymatic defence systems
Rectal Adenocarcinoma	Stimulation of ROS generation leading to dissipation of mitochondrial membrane potential, caspases cascade and apoptosis
Oral Squamous Carcinoma	Mediation of mitochondrial pathway and apoptosis
Hepatocellular Carcinoma	Interaction of CYP1A1 enzyme, mediating deficiency of benzo(a)pyrene (BP) metabolism and consequently abolishing cancer aggressiveness
Breast Cancer Stem Cells	Inhibition of Wnt/beta-catenin signalling pathway; repression of mammosphere formation

4.4 Immunomodulatory Activity

Piperine shows remarkable potential for its immunomodulatory properties. The 5-fluorouracil (5-FU) incubation of tumour cell lines in the presence of piperine resulted in increased growth inhibition, observed by decreased 5-FU IC50 values. Additionally, piperine administration reduced the leukopenia caused by 5-FU treatment, demonstrating better immunocompetence previously inhibited by 5-FU. According to the study of Bernardo et al., piperine suppresses the proliferative response brought on by lipopolysaccharide (LPS) and immunoglobulin-IgM

antibody *in vitro*. Additionally, piperine decreased the extent of CD86 and blocked IgM antibody production. Furthermore, piperine-activated p38 and JNK MAPK when combined with gamma-aminobutyric acid (GABA), raised the extent of EPO and EPO-R and up-regulated the activity of NF-B and IL-10 (Stojanović-Radić et al., 2019).

Additionally, piperine may be thought of as a strong immunomodulator that inhibits inflammation of the airway in a mouse asthma model through increased TGF-beta gene expression in the lungs (Shityakov et al., 2019). The inhibition of Th2 cytokines (IL-4, IL-5, and IL-13), immunoglobulin E, and eosinophil CCR3 expression in the lungs also contributes to the substantial inhibitory effects on inflammation of the airway in a mouse model of asthma. For this reason, it can be thought of as a potential immunomodulator by suppressing Th2 cytokines (Gorgani et al., 2017).

4.5. Anti-inflammatory Activity

The anti-inflammatory properties of black pepper were initially revealed about 20 years ago. According to Mujumdar et al. (1990), piperine inhibits acute inflammation by activating the pituitary-adrenal axis. Later, through various in vitro studies, Bang et al. (2009) improved the anti-inflammatory properties of piperine (20 and 100 mg/kg/day). They proposed plausible mechanisms for their claimed characteristics, including suppression of interleukin, matrix metalloproteinase, prostaglandin E2, and activator protein 1. Piperine (50/100 ug/ml) decreased the level of β-glucuronidase and lactate dehydrogenase in a dose-dependent manner, according to a recent study by Sabina et al. (2011). The expression of some enzymes, such as 5-lipoxygenase and COX-1, which are in charge of leukotriene and prostaglandin production, can be restricted by piperine and a few other substances. Together, these actions are beneficial for preventing rheumatoid arthritis and other degenerative diseases (Butt et al., 2013).

It was discovered that black pepper and its active components have anti-inflammatory properties based on carrageenan-induced paw oedema using a plethysmometer. Piperine demonstrated an anti-inflammatory effect in this trial at doses of 10 and 15 mg/kg after 30 minutes, lasting for 60 minutes, while hexane and ethanolic fruit extracts also demonstrated activity at a lower level of 10 mg/kg, lasting for 120 minutes. Inhibiting endotoxins triggered by LPS, piperine showed activity in synoviocytes that activate interleukin (Lipopolysaccharides). Additionally, it was

shown that piperine, at concentrations between 10 and 100 g/ml, inhibits the synthesis of prostaglandin E, MMP-13, and IL-6. Additionally, the anti-inflammatory potential of piperine was examined in colorectal locations, where it was found to reduce ulcerative colitis brought on by acetic acid and FFA (free fatty acid)-induced inflammation, which is mediated by TLR4 (Toll receptor). Likewise, other black pepper alkaloids reduce inflammation in murine macrophages by prohibiting the NF-kB pathway from being activated (nuclear factor kappa-light-chain-enhancer of activated B cells). A very substantial difference was seen in comparing the black pepper essential oil to the common medication Diclofenac. Black pepper essential oil inhibited paw oedema in the chronic inflammation caused by formalin by 50% at 500 mg/kg, compared to 57.5% at 10 mg/kg with Diclofenac (Milenković & Stanojević, 2021).

4.6. Anti-diarrheal Activity

Along with the antibacterial properties already mentioned, black pepper also fights against some bacteria that cause diarrhoea. Black pepper aqueous extract exhibits strong dose-dependent antimotility, antisecretory, and antidiarrheal effects at doses of 75, 150, and 300 mg/kg. The presence of alkaloids in black pepper is what induces this effect (Saleem et al., 2022).

Piperine reduces intestinal fluid build-up in the mouse intestine and helps avoid diarrhoea caused by various chemicals and oils. The P. nigrum's active ingredients stimulate the synthesis of proteins that are later utilized for the assembly of the cytoskeleton system due to surface adsorption property and permeate the uptake of various amino acids through membrane activation in the rat jejunum epithelial cells. Capsaicin-sensitive neurons were responsible for controlling the decrease in fluid secretion and accumulation (Ahmad et al., 2012).

4.7. Anti-asthmatic Activity

Due to piperine's capacity to inhibit phosphodiesterase (PDE) and block Ca2+ channels, black pepper can be used to treat asthma. Also, piperine can activate opioid receptors, which explains black pepper's anti-tussive characteristics and its long-standing use for a persistent cough (Rehman et al., 2015).

Piperine can also inhibit the PDE enzyme. PDE inhibition has a variety of anti-inflammatory actions on the many asthma-causing cells. Depending on the concentration, piperine displays a

variety of behaviours. The study discovered that in guinea-pig tracheal tissues, PDE inhibition is most pronounced at 3-30M concentration. On the other hand, Ca2+ channel blockage in bovine coronary artery preparations was most pronounced at 30-300M. Piperine also primarily stimulates opioid receptors between 300 and 1000M. (Rehman et al., 2015).

4.8. Analgesic

The tail immersion, analgesy-meter, hot plate, and acetic acid-induced writhing tests were used to determine the analgesic activity of hexane and ethanolic extracts of *Piper nigrum* and its constituent piperine. The ethanol extract was most effective in the analgesy-meter test after a dose of 10 mg/kg given after 60 minutes, whereas piperine showed the greatest analgesic effect after 120 minutes at a dose of 5 mg/kg. In the hot plate method, piperine showed the fastest reaction time for licking or jumping paws (12.870 s after 30 min at a dose of 10 mg/kg). Piperine (10 mg/kg) and the ethanol extract (15 mg/kg) fully halted the number of writhes in mice induced by acetic acid in the writhing test (Takooree et al., 2019).

Piperine was tested for its in vivo analgesic effects on mice. Piperine's analgesic efficacy was assessed using mouse models for acetic acid-induced writhing and tail flicking. Intraperitoneal administration of piperine at specific doses (30, 50, and 70 mg/kg) compared to indomethacin at a dose of 20 mg/kg showed a significant ($P<0.01$) reduction in writhing in mice induced by acetic acid (Damanhouri, 2014). By inhibiting the cyclooxygenase enzyme in peripheral tissues and preventing signal transduction at primary afferent pain receptors, aspirin and other nonsteroidal anti-inflammatory medications can lessen convulsions in the mouse model described above. Therefore, the inhibition of local prostaglandin levels may be the cause of piperine's analgesic effects. This demonstrates that both peripheral and central modes of action contribute to piperine's analgesic effects (Milenković & Stanojević, 2021).

4.9. Digestion Enhancer

Traditionally, people have used black pepper and its active components as treatments for stomach disorders. It is generally acknowledged that black pepper helps to both prevent and treat digestive issues. By stimulating histamine H2 receptors, black pepper rises the amount of hydrochloric acid produced by the stomach, hence enhancing digestion. Piperine is absorbed by

about 44% to 63% from the mucosal side, as proved by the presence of the substance in intestinal tissue and serosal fluids. Piperine promotes gastrointestinal tract function by improving enzyme synthesis, increasing nutritional absorption, and boosting defence mechanisms. Examples include improved intestinal absorption of trace elements (selenium), vitamins B, and β-carotene, as well as methionine and calcium ions (Butt et al., 2013).

According to Ahmad et al., piperine boosts gastric and salivary secretions and the generation and activation of salivary amylase (Srinivasan, 2007)**.** Black pepper secures the gut from gastric secretions and ROS damage through its antioxidant properties. Additionally, it boosted the activity of antioxidant enzymes such as glutathione reductase, catalase, superoxide dismutase, and glutathione-S-transferase. Together, these systems regulate mucosal health to provide gastro-protection (Khan et al., 2021). By stimulating pancreatic enzymes, dietary *Piper nigrum* improves digestion and significantly reduces the transit time taken by food through the digestive tract (Saleem et al., 2022).

Piperine enhances nutrient absorption in various ways, including by increasing the creation of micelles and altering the membranes of epithelial cells due to piperine's affinity for fatty substances. Additionally, ultrastructural studies utilizing electronic scanning microscopy showed that the size of the microvilli increased due to piperine and thus increased intestinal mucosal permeability (Jogdand & Singh, 2021)**.**

Due to its capacity to inhibit human CYP3A4 and P-glycoprotein (P-gp), two important regulators of xenobiotic metabolism, piperine is widely acknowledged as a bioavailability enhancer. The compound piperine enhances the bioavailability of xenobiotics by blocking metabolism and efflux (Chavarria et al., 2016). The active ingredient in turmeric, curcumin, which aids in the reduction of inflammation, is a prime example of this. According to research discovered in Planta Medica (1998), piperine improved the rate of absorption and serum levels of curcumin in both rats and people without causing any side effects (Dahmani et al., 2019). However, piperine itself has a low bioavailability that can be improved by combining it with ethyl oleate, Tween 80, and Transcutol P as a self-emulsifying drug delivery system in intestinal absorption models (Shityakov et al., 2019).

4.10. Hepatoprotective

Ethanol-CCl4 administration caused a significant increase in triglycerides, alanine transaminase, aspartate transaminase, alkaline phosphatase, and bilirubin levels while causing a remarkable decrease in superoxide dismutase, catalase, and glutathione reductase levels. These levels then became normal following pre-treatment with a methanolic extract of *Piper nigrum* and piperine at predetermined dosages. Moreover, lipid peroxidations were also significantly reduced (Damanhouri, 2014).

Piperine was used to treat the d-galactosamine-induced liver injury model in mice, which normalizes serum levels of glutamic oxaloacetic transaminase and pyruvic transaminase. This mechanism was related to the hepatocytes' decreased sensitivity to TNF-α (Shityakov et al., 2019)**.** In numerous models of liver damage, the proinflammatory cytokine TNF-α sign plays a noteworthy role in controlling hepatocyte proliferation, tissue healing, and matrix remodelling. TNF-α causes cellular apoptosis, which in turn mediates a range of organ damage. The biological effects of TNF-α have been linked to alcohol, viral hepatitis, ischemia/reperfusion, and hepatic injury-induced hepatic toxins in the liver. One of the initial processes in the hepatic inflammatory response, which results in cytotoxicity, hepatocyte apoptosis, and necrosis, is the generation of TNF-α. TNF-α can therefore be the key target for the development of hepatoprotective drugs. The fact that acetaminophen raises blood TNF-α levels has been shown to point to this cytokine's involvement in acetaminophen-induced hepatotoxicity. But in mice treated with acetaminophen, piperine treatment greatly decreased the increased TNF-α. Thus, it can be concluded that *Piper nigrum* has hepato-protective properties and considerable medicinal potential for treating liver diseases (Sabina et al., 2010).

4.11 Antidepressant Activity

The antidepressant activity of piperine and its potential mechanisms were examined in a corticosterone-induced depressive mice model. After receiving corticosterone injections for three weeks, mice began to exhibit depressive-like behaviours. The depression was confirmed by forced swim test and tail suspension test results that showed a substantial decrease in sucrose consumption and an increase in immobility time respectively. Furthermore, mice treated with corticosterone also had considerably lower levels of mRNA and protein for brain-derived

neurotrophic factors in the hippocampus. According to Bai et al., corticosterone caused behavioural and biochemical alterations in mice treated with piperine. These findings denoted that piperine had an antidepressant effect in depressive mouse models brought on by corticosterone (Kumar Srivastava & Kumar Singh, 2017).

4.12 Anticonvulsant Activity

Belemkar, Kumar, and Pata (2013) found that both the maximal electroshock seizure (MES) induced model and the pentylenetetrazol (PTZ) induced model showed suppression of the initiation and duration of seizures in Wistar rats. Additionally, Bukhari et al. (2013) discovered that treating mice with 50 mg/kg of piperine was more beneficial than treating them with 70 mg/kg in preventing PTZ-induced seizures. The latency of convulsions caused by picrotoxin increased to 878.5 s at the maximum tested dose of piperine (70 mg/kg), compared to 358.4 s in the control group (Takooree et al., 2019)**.**

4.13 Other Pharmacological Activities

Piperine and black pepper inhibit the absorption of cholesterol and promote the movement of cholesterol transporter proteins. It enhances digestion by accelerating the breakdown of fat molecules into more easily absorbed simple molecules and reduces the build-up of fat in the body (Dahmani et al., 2019). With a high-fat diet, piperine from *Piper nigrum* (40 mg/kg) substantially reduced body weight, total cholesterol, triglycerides, LDL, VLDL, and fat mass while raising HDL levels (Saleem et al., 2022). Increased levels of hepatic cholesterol 7 alpha-hydroxylase (CYP7A1), lipoprotein lipase (LPL), LDL receptor (LDLR), and plasma lecithin cholesterol acyltransferase (LCAT) may be responsible for this, which would further increase cholesterol excretion (Wang et al., 2021).

Piperine also shows notable anti-allergic action in mice suffering from allergic rhinitis brought on by ovalbumin. Piperine considerably reduced the sneezing, redness and rubbing brought on by nerve endotoxin caused by histamine generated as a response to an antigen-antibody reaction. However, piperine also decreased levels of nitric oxide (NO) because fewer eosinophils migrated into the nasal epithelial tissue (Stojanović-Radić et al., 2019). By inhibiting voltage-dependent Ca2+ channels, reducing elastin formation, and elevating NO, it reduced mean artery pressure.

Additionally, it was seen that the angiotensin-1 converting enzyme (ACE), which transforms angiotensin-I into angiotensin-II, a strong vasoconstrictor, was blocked in vitro by the essential oil of black pepper (Wang et al., 2021). The capacity of the essential oil to inhibit ACE activity in a concentration-dependent manner in vitro suggests that it might be able to modify the conversion of angiotensin-I to angiotensin-II which is responsible for hypertension (Oboh et al., 2013).

In subacute experimental models, piperine's impact on blood glucose levels was examined in diabetic rats produced by streptozotocin. In diabetic rats induced by streptozotocin, the results imply that piperine at a dose of 50 mg/kg exhibited antidiabetic action (Dahmani et al., 2019). D-limonene, the primary active ingredient, has preventative and ameliorative effects on insulin resistance and dyslipidemia in other animal model investigations (X. Han et al., 2018). Furthermore, crude extracts and ethyl acetate extract obtained from black pepper decreased the activity of cholinesterase, raising the possibility that black pepper or one of its components could be utilized to treat Alzheimer's disease (Wang et al., 2021).

Chapter 5

Effect on Metabolic Enzymes

Piperine has an enzyme-inhibiting molecular structure that is required for a wide extent of metabolic enzymes, including cytochrome NADPH (nicotinamide adenine dinucleotide phosphate), CYP3A4 (cytochrome P450 Family 3 Sub-family A Member 4), AHH (aryl hydrocarbon hydroxylase), UDP-GDH (uridine diphosphate glucose dehydrogenase) and UDPGT (Uridine Diphosphate-glucuronyl-transferase (Milenković & Stanojević, 2021).

The hepatic cytochrome P450 levels of rats and the activities of benzphetamine N-demethylase, aminopyrine N-demethylase, and aniline hydroxylase significantly decreased after receiving piperine intraperitoneally or intragastrically at doses of 100 mg/kg or 800 mg/kg, respectively. The inhibition of D-aminolaevulinic acid synthase, which resulted in a drop in hepatic heme, or, less likely, the activation of heme oxygenase activity, may have all contributed to the decrease in cytochrome P450 and other components of the combined oxidase system. However, when the intragastric dose was reduced to 100 mg/kg, the activities of hepatic microsomal cytochrome P450 and cytochromeb5, NADPH-cytochrome-C reductase, benzphetamine N-demethylase, aminopyrine N-demethylase, and aniline hydroxylase were greatly increased in rats. These findings imply that, based on the dosage and route of administration, piperine has both inhibitory and stimulatory effects on the cytochrome P450-mediated activities of the microsomal monooxygenases (H. K. Han, 2011). There is proof that piperine inhibits MAO-A and MAO-B (Monoamine oxidase inhibitors A and B) in a competitive, reversible, and nonselective manner. Tyr326, Tyr398, and Tyr435 are the amino acids that piperine usually binds to. These amino acids are the binding sites for MAO-B enzymes. Tyr435 and Tyr398 (Tyrosine residues) are crucial for assisting MAO-B enzymes in deaminating. Black pepper extracts have an inhibiting effect on the CYP (Cytochrome P450) family enzymes CYP1A2, CYP2C9, CYP2D6 and CYP3A4. Even though there have only been a few types of research, piperine has been found to inhibit P450 enzymes. Piperine is a less effective inhibitor of CYP1A2 and CYP2D6 and a non-competitive inhibitor of CYP3A4 (Milenković & Stanojević, 2021).

Piperine considerably lowered the activity of both UDP-glucuronyl transferase (UGT) and liver microsomal aryl hydroxylase. It substantially reduced hepatic microsomal UGT activity in vitro

by 36%. Additionally, piperine showed a 26% reduction in the hepatic microsomal NADPH-cytochrome c reductase activity. According to Atal et al., oral piperine treatment in rats severely reduced both the hepatic AHH and UGT activities. At doses of 10 and 25 mg/kg, the activity of AHH in rats was decreased by 50 and 80%, respectively, and the action of UGT was inhibited by 36 and 55% at these corresponding concentrations (H. K. Han, 2011).

In rats and guinea pigs, the effects of piperine on hepatic and intestinal UDP-GDH and glucuronidation potentials were investigated. Through non-competitive inhibition, piperine caused UDP-GDH to be strongly inhibited (by 50% at 10 μM) in the liver and gut. However, enterocytes isolated from the small intestines of guinea pigs responded to piperine more favourably than rat hepatocytes in the aspect of lowering the UDP-glucuronic acid (UDPGA) levels. UDPGA content reduction by piperine may be caused by the inhibition of UDP-GDH, a crucial enzyme involved in UDPGA synthesis. This is because the rate of glucuronidation in situ is influenced by the absolute amount of UGT and its cofactor, UDPGA. These findings indicated that piperine exerts higher effects on intestine glucuronidation compared to rat liver and that piperine is a strong inhibitor of UDP-GDH. The brush boundary membrane enzymes glycyl-glycine dipeptidase, leucine amino peptidase, g-glutamyl transpeptidase, and alkaline phosphatase were also induced by black pepper and piperine in the jejunal mucosa. (H. K. Han, 2011)**.**

Both COX-1 and COX-2 enzymes, which are cyclooxygenases, can be inhibited by piperine. Piperine, piperonal, piperic acid, and piperonyl acid have been shown to have LOX (Lipoxygenase) inhibitory action using LOX inhibition studies. It was discovered that piperonyl acid had a greater inhibitory power (79%) than piperic acid (69%), and piperonal acid (64%), and when comparing the results of enzymatic testing in equimolar combinations (Milenković & Stanojević, 2021)**.**

Chapter 6

Enhancement of Drug Bioavailability

The bioavailability of several medications is enhanced by black pepper. For instance, piperine improves the absorption of the medications used for treating epilepsy such as carbamazepine and phenytoin and hence increases their oral bioavailability. Piperine also enhanced the bioavailability of nimesulide, an anti-inflammatory drug (Butt et al., 2013). Furthermore, piperine considerably increased the oral exposure of fexofenadine (an anti-histamine medication), probably by inhibiting P-gp-mediated efflux in the time of intestinal absorption. However, piperine's continuous usage had no effect on the drug's intravenous pharmacokinetics (Jin & Han, 2010).

Antiretroviral medications and piperine are combined for treating HIV-1 infections. In the Pattanaik et al. trial, an improvement in carbamazepine bioavailability was noted following just a single 20 mg dosage of piperine. Due to the suppression of the CYP2C9 enzyme by piperine, the half-life of diclofenac was extended by 34%. According to Auti et al., whether piperine was given solely or in combination with a certain dose of piperine (10 mg/kg), it significantly altered the pharmacokinetic profile of secnidazole (10 mg/kg), simvastatin (10 mg/kg) and verapamil (10 mg/kg). The bioavailability of these medicines was enhanced by piperine by 2.53, 1.55, and 1.08 times, respectively (Milenković & Stanojević, 2021).

Piperine increased the bioavailability of various antibiotics, including norfloxacin, and β-lactam antibiotics such as amoxicillin, ampicillin and cefotaxime sodium. Their increased bioavailability occurs due to piperine's inhibition of the enzymes in the liver that are responsible for these antibiotics' metabolism. Rifampicin and piperine remarkably improved the antibiotic's ability to inhibit Mycobacterium smegmatis compared to rifampicin alone (Gorgani et al., 2017). Recent research has shown that piperine increases the bioavailability of anti-cancer medications such as docetaxel, etoposide, paclitaxel, 5-fluorouracil and rapamycin both in vitro and in vivo (Zadorozhna et al., 2019). Recently, the most crucial therapy for metastatic castration-resistant prostate cancer has been docetaxel, a cytotoxic chemotherapy drug (CRPC). Piperine co-administration improved the anticancer activity of docetaxel in a xenograft model of human CRPC.

Additionally, piperine was used to enhance the bioavailability of acyclovir. Acyclovir-loaded floating microspheres were created using the emulsification solvent evaporation method, and the impacts on bioavailability due to incorporating piperine on acyclovir were examined. Results demonstrated that compared to the drug solution or piperine-free microspheres, combining microspheres with piperine enhanced the relative bioavailability of acyclovir (Gorgani et al., 2017)**.**

Piperine also improves the absorption of both herbal and synthetic medications, including curcumin and resveratrol. By blocking resveratrol's metabolism and lowering the dosage of resveratrol needed in a clinical setting, studies have examined that piperine significantly boosted resveratrol's in vivo bioavailability (Gorgani et al., 2017)**.**

Chapter 7

Conclusion and Future Perspectives

Despite the fact that piperine has many therapeutic qualities, its biomedical applications are still restricted for its low bioavailability and low water solubility. Piperine supramolecular formulation with some hydrophilic ingredients, such as unaltered cyclodextrin excipients, can help to improve this problem (Shityakov et al., 2019).

The main cause of piperine's low bioavailability is likely its hydrophobic properties and low water solubility. The poor water solubility of piperine impacts the rate-limiting phase in its absorption process, which is the main obstacle to its development as a medication from the lab to the clinic. This necessitates the creation of novel formulations to improve piperine's water solubility and increase its bioavailability. There has been very little research in this area, and the literature is lacking in findings discussing formulations of piperine for therapeutic uses (Gorgani et al., 2017).

A few approaches investigated for enhancing the bioavailability of piperine are discussed below:

1. Nanoparticles

The solvent evaporation approach produced piperine-loaded poly (ethylene glycol)-poly (lactic-co-glycolic acid) nanoparticles that were stable in aqueous solutions. Additionally, if piperine was administered at larger doses, these nanoparticles could boost the bioavailability of the compound and lessen its toxicity due to a decreased metabolic rate (Gorgani et al., 2017).

2. Liposomes

Polymers and liposomal encapsulation were used to mask piperine's pungent smell. When given intranasally, piperine could penetrate the brain. Compared to an oral dose, intranasal piperine liposomes delivered their substance to the hippocampus at a higher pace and to a greater degree. The dose of piperine administered intranasally using liposomes may be reduced. (Gorgani et al., 2017).

3. Hot melt extrusion

The permeability, solubility, and oral absorption of piperine can be improved with hot melt extrusion (HME). Permeability tests revealed an increase in piperine absorption of 10% (w/w) piperine/Soluplus extrudate than pure piperine. These findings show that piperine's bioavailability and absorption can be enhanced by HME (Gorgani et al., 2017).

4. Self-emulsifying drug delivery systems

Compared to the self-made capsules, the advanced SEDDS formulation's oral bioavailability increased by 625.7%. Additionally, the SEDDS greatly excelled over the self-prepared capsules in terms of the piperine release rate. The rapid release of piperine from the SEDDS formulation may be due to the spontaneous emulsion formation and small droplet size. In studies on intestinal absorption, the SEDDS formulation's permeability was higher compared to the reference control (Gorgani et al., 2017)**.**

5. Microspheres

The hepatoprotective and antiulcer properties of floating and mucoadhesive gastro-retentive piperine micro-spheres were studied using the solvent evaporation method. The outcomes showed that, in comparison to other formulations, floating piperine microspheres provided higher protection against stomach ulcers. They outperformed mucoadhesive microspheres in terms of hepatoprotective efficacy, perhaps because of their smaller particle size. Compared to free piperine, the two piperine microspheres created demonstrated stronger hepatoprotective and antiulcer properties (Gorgani et al., 2017).

One of the most efficient approaches for enhancing the protection and production of black pepper is the biotechnology approach. Black pepper production can be increased by using specific varieties and effective agricultural methods. Nematodes, bacteria like xanthomonas campestris pv. eticola, nematodes, and fungi like *Phytophthora capsici* or *Colletotrichum gloeosporioides* are the most frequent diseases of black pepper. In order to reduce physical,

chemical and microbiological hazards during the processing of black pepper, good manufacturing practices (GMP) should be followed, in accordance with international standards set by the FAO/WHO. This will ensure that the ultimate product has greater nutritional value.

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