

Review Article

Therapeutic potential of bioactive compounds of *Piper nigrum* L. (Black Pepper): A review

Abstract

The present study highlights the Bio activities and Pharmacology of *Piper nigrum* L. (Black Pepper). *Piper nigrum* L is commonly known as Kali Mirch, Pippali, Milagu Peppercorn, White pepper, Green pepper, Black pepper, and Madagascar pepper longum plant is a deciduous slender aromatic climber. The major source of bioactive constituents in black pepper is present in the fruits. It contains major pungent alkaloid piperine (1-peperoyl piperidine) which is known to possess pharmacological actions. Antimicrobial activity of black pepper evaluated the antibacterial potential of aqueous decoction of *Piper nigrum* L. (black pepper), against different bacterial isolates from oral cavity of individual volunteers.

Keywords: black pepper, Pharmacology, aromatic climber, alkaloid, medicinal agent

1. INTRODUCTION

Piper nigrum (Black pepper), is called as "The King of spices" and is used as a common spice among various spices (Mathew PJ et al., 2001, Srinivasan et al., 2007). It is used as a medicinal agent, a preservative, and in perfumery (Singh VK, et al., 2014). Furthermore, researchers suggest that the consumption of black pepper, due to the presence of the numerous bioactive compounds, exhibit several pharmacological activities with human dietaries, medicine, preservative, and biocontrol agents (Awen BZ, et al., 2010, Hussain A et al., 2011). "The major source of bioactive constituents in black pepper is present in the fruits. It contains major pungent alkaloid piperine (1-peperoyl piperidine) which is known to possess pharmacological actions (Ahmed N et al., 2015) reported that this plant and its active components piperine when orally administered, it can kindle the digestive enzymes of pancreas and intestine and also increases biliary bile acid secretion". It is used as medicine

for digestive disorders like large intestine toxins, different gastric problems, diarrhea, indigestion respiratory disorders including cold fever, and asthma[11-13](Sujata R et al., 2003, Parganiha R, et al., 2011, Fan LS, et al., 2011).

“Piper nigrum L is commonly known as Kali Mirch, Pippali, Milagu Peppercorn, White pepper, Green pepper, Black pepper, and Madagascar pepper **longum plant is a deciduous slender aromatic climber. Structurally, it has perennial woody roots or a perennial creeping shrub that belongs to the family Piperaceae. Structurally it is Erect, glabrous with swollen nodes, roots clasping at nodes which help it to attach to the host trees”** (Patel et al., 2022; Umang and Ankita, 2014; Vyas et al., 2010; Zaveri et al., 2010). Leaves are ovate shape, an **arrangement is alternate, apex acute to acuminate with entire margin** (Manoj P, et al., 2004), while the Fruits have Spikes which are long cylindrical, oblong, berries red or black when ripe, globose with aromatic odor and pungent taste. (Sumy O, et al., 2000, Viswanathan TV et al., 1995, Chadha KL et al., 1987). The flowers are, biologically male or female and are born on different plants. *“Piper nigrum L* is grown in the tropical rain forests of India, Nepal, Indonesia, Malaysia, Sri Lanka, Timor, and the Philippines. It also occurs in hotter parts of India, from the central Himalayas to Assam, **Khasi and Mikir hills, lower hills of West Bengal, and evergreen forests of the Western Ghats from Konkan to Kerala and Nicobar Islands”**. (Satyavati G, et al., 1987)

2. Bioactive constituents from *Piper nigrum*

The major bioactive compounds in pepper are Phenolics, various derivatives of lignans, terpenes, chalcones, flavonoids, alkaloids, and steroids (Parmar Vs et al., 1997). the bioactive compounds were isolated from the *P. nigrum* fruits. The major constituents of seeds include

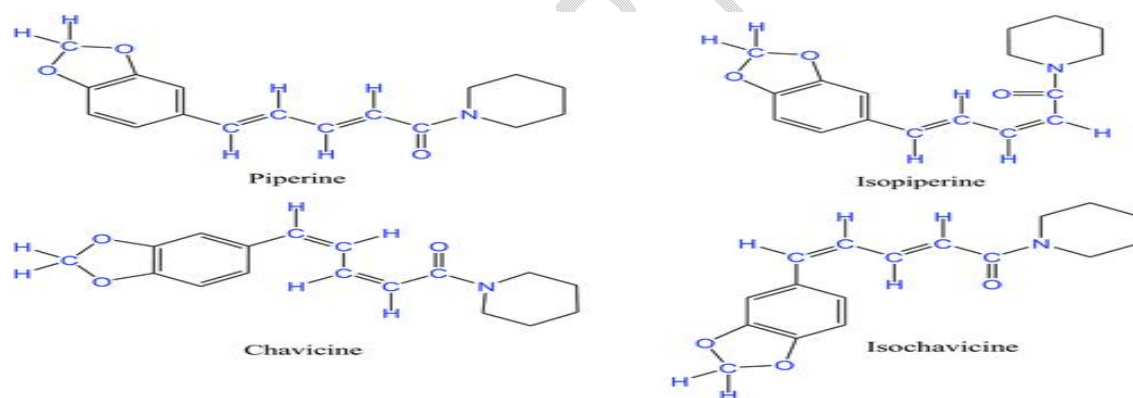
Dihydropiperidine (Parmar Vs et al., 1997), (2E, 4E)-N-Eicosadienoyl piperidine (Molteni R, et al., 2006) Guineensine (Lee Cs et al., 2006), pentadienoyl as piperidine, (2E, 4E)-Nisobutyldecadienamide, isobutyl-eicosadienamide (Lee SA et al., 2005), isobutyl-eicosatrienamide (Kumar A et al., 2008), isobutyloctadienamide (Parmar Vs et al., 1997), Piperamide Chonpathompikunlert P,et al., 2010) Piperamine, (Parmar Vs et al., 1997), Piperettine (Ramji MT et al, 2011), Piperidine ,(Kolhe SR, et al., 2011) Piperine (Kokate CK , et al., 2008), Piperolein B, Trichostachine, Sarmentine, Sarmentosine, Tricholein,

Retrofractamide A ,(Mann A. et al., 2011). The active agent of piper species was the first compound isolated from different species in family Piperaceae (Ahmed N, et al., 2011).



Image 1: Black pepper (*Piper nigrum* L.)

Important structural aspects of the bioactives from *Piper nigrum* L



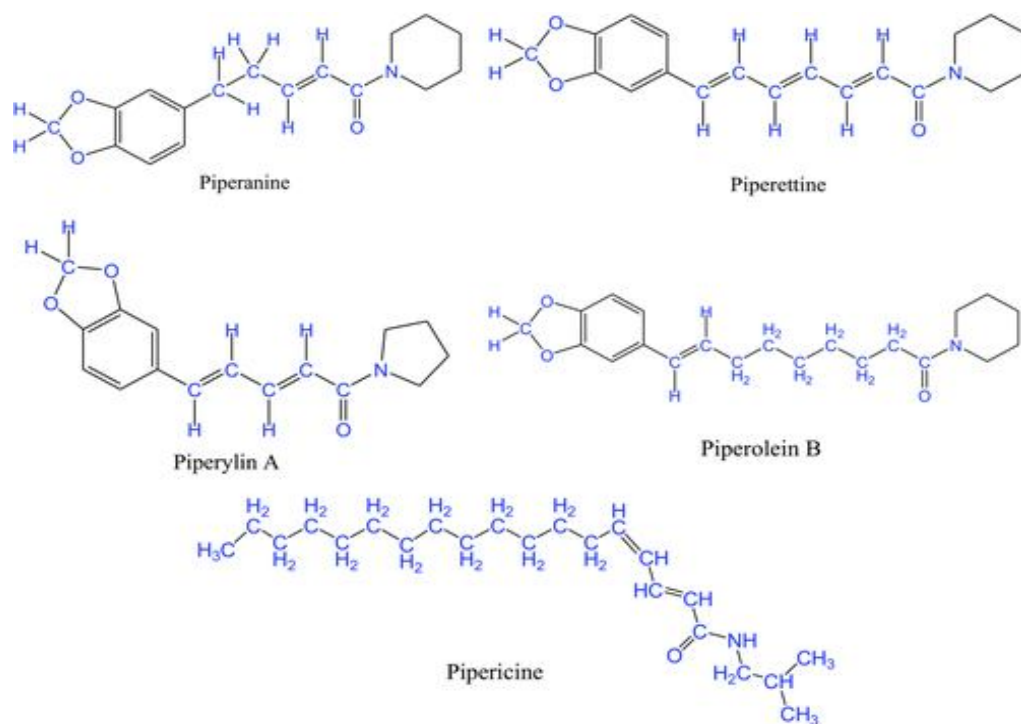


Figure 1: Bioactive compounds from *Piper nigrum L*

3. PHARMACOLOGY

3.1. Antioxidant activity

“Plants are an important source of antioxidants. Some in vitro studies revealed that Piperine is known to possess protective effects against oxidative damage due to the inhibition property of free radicals and reactive oxygen species. Many studies were done on Piper nigrum and it was found to prevent oxidative stress by inhibiting lipid peroxidation, human lipoxygenase, and arresting hydroxyl and superoxide free radicals, decreasing carcinogenesis in animal studies. The memory enhancing and antioxidant proprieties of the methanolic extract of Piper nigrum L. fruits were investigated in Alzheimer’s disease model in rats”. (Vijayakumar RS et al., 2004) (Selvendiran K et al., 2004). (Ahmad N, et al., 2010) “The amyloid beta (1-42)-treated rats showed the diminishing of spontaneous star variation percentage within Y- maze

task and enhancement of work memory and reference memory errors within the radial arm-maze task by measuring the total content of reduced glutathione, malondialdehyde, and protein carbonyl levels in the hippocampus the antioxidant activity was evaluated by measuring activities of glutathione peroxidase, catalase, superoxide dismutase. Injecting the methanolic extract of *Piper nigrum* significantly improved memory performance and exhibited antioxidant potential. Many studies suggest that methanolic extract of *Piper nigrum* ameliorates amyloid beta (1-42)-induced spatial memory deterioration by depletion of the oxidative stress in the hippocampus of rats” (Hritcu L, et al., 2014)

3.2. Anticancer activity

Various studies have shown that flavonoids may serve as a protective role in breast cancer prevention (Cooray, et al., 2004; Marchand et al., 2002; Rodgers, et al., 1998). “Ethanol extract of peppercorn and piperine showed effective immunomodulatory and antitumor activity” (Damanhoury ZA et al., 2014). Piperine has been found to hinder the proliferation and survival of various cancerous cell lines via modulating cell cycle development and showing anti-apoptotic activity. The piperine can be considered as a worthy agent for controlling angiogenesis in the Prostate of men. “Research has presented that piperine has good anticancer activity against prostate cancer cells of both androgens dependent and independent. Piperine can modulate lipid peroxidation and activation of antioxidative protection enzyme, thus reducing lung cancer” (Mona AM et al., 2009)

3.3. Antimicrobial activity

“Antimicrobial activity of black pepper Khan and Siddiqui in 2007 evaluated the antibacterial potential of aqueous decoction of *Piper nigrum* L. (black pepper), against different bacterial isolates from oral cavity of individual volunteers. The silver nanoparticles from leaf and stem extract of *Piper nigrum* showed excellent antibacterial activity against plant pathogens. The research findings showed the strongest antibacterial activity of Black pepper (aqueous decoction) at the concentration of 10µL/disc” (Khan M, et al., 2007). In a recent study “antibacterial activity of the synthesized silver nanoparticles of *Piper nigrum* was evaluated against agricultural plant pathogens. Authors confirmed that the antibacterial activity of silver nanoparticles is a beneficial application in crop improvement and protection in agricultural nanotechnology” (Reddy Srinivas et al., 2001)

The aqueous seed extract of *Piper nigrum L* demonstrated an active antibacterial inhibitory effect against *Staphylococcus aureus*, and *Bacillus subtilis*. The research findings showed that the strongest antibacterial activity of Black pepper (aqueous decoction) at the concentration of 10 μ L/disc (Khan M, et al., 2007) on against agricultural plant pathogens. The silver nanoparticles from leaf and stem extract of *Piper nigrum* showed excellent antibacterial activity against plant pathogens (Reddy Srinivas et al., 2001)

3.4. Anti-inflammatory activity

piperine isolated from *P. nigrum* possesses potent anti-inflammatory analgesic and antipyretic activities. It inhibits the adhesion of endothelial monolayer to neutrophils and due to such inhibitory activity the tumor necrosis factor-alpha-induced expression of cell adhesion molecules was blocked i.e. intercellular adhesion molecule-1, vascular cell, and E-selectin. (Vijayakumar RS et al., 2004) also reported “another blocking system that piperine blocks the phosphorylation and degradation of I κ B α by attenuating tumor necrosis factor-alpha-induced I κ B kinase activity”. (Singh and Duggal et al., 2009) also documented that GM-CSF, IL-6, TNF-alpha, and IL-1 beta which was proinflammatory cytokines were dramatically reduced by the administration of piperine.

The in vitro anti-inflammatory activities were also evaluated on interleukin 1 β stimulated fibroblast-like synoviocytes obtained from rheumatoid arthritis. The prostaglandin E2, cyclooxygenase 2, interleukin 6, and matrix metalloproteinase levels were evaluated by ELISA and RT-PCR methods of analysis. Piperine was found to reduce the synthesis of prostaglandin E2 component at concentrations of 10-100 μ g/mL.

3.5. Hepatoprotective activity

“Aqueous extract of fruits of *P. longum* and piperine were selected to study their hepatoprotective potential on administration with normal doses of anti-tubercular drugs” (Gurumurthy et al., 2012). *P. longum* and piperine on administration with the anti-TB drugs lowered the rate of lipid peroxidation and also increased the reduced glutathione levels and thus exhibits the hepatoprotective effect

“When experimental mice with D-galactosamine induced liver toxicity were exposed to dose-dependent piperine, it inhibited an increase in serum GPT and GOT levels and suggested that this inhibitory effect depended on the reduced sensitivity of hepatocytes to tumor necrosis factor” which is observed by (Matsuda et al., 2008)

3.6. Cosmoperine activity

cosmoperine prepared from piperine used in cosmetics, (Sabina Corporation et al., 2011) reported that a natural bio-enhancer improves the penetrability of active compounds through the skin. Cosmoperine activates and kindles the natural power of the skin to absorb nutrients (Badmaev V et al., 1999, Majeed M et al., 2005). Cosmoperine isolated from piperine is nonirritant, interacts with the skin quantitatively and qualitatively in various means, and also, cosmoperine is pain relieving and causes skin blushing due to vascular puffiness as well as a slight skin prickling sensation.

3.7. Antifertility activities

Garg in 1981 showed (Garg SK et al., 1981) at root extract of *P. longum* when used along with *Embelia ribes* seeds showed 100% anti-fertility activity in female albino rats. It is supposed that *P. longum* probably potentiates the contraceptive activity of other plant products, the probability of such a combination needs to be investigated further for the development of a contraceptive for the female without interfering with the activity of ovarian hormones on uterus (Munshi SR et al., 1972) reported in Ayurveda *Garbhanivarana Aushadham* which is used for both female and male

3.8. Antidepressants activities

“The aqueous extract of piperine was evaluated in a corticosterone-induced model of depression in mice.. The depression was observed by the significant decline in sucrose utilization and augmentation in immovability time in the Corticosterone-induced behavioral and biochemical changes were pointedly weakened after treatment to animals with Piperine. These results exhibited that piperine produces an antidepressant-like effect in a corticosterone-induced model of depression in mice” (Mao QQ et al., 2014)

3.9. Antidiabetic/ anti-hyperglycemic activity

In induced diabetic rats model, *Piper longum* root aqueous extract and its constituents, (PlrAqe) in streptozotocin (STZ) (Nabi et al., 2013) suppresses insulin levels and liver glycogen. This study predicts that the plant extract is capable of managing hyperglycemia and complications of diabetes in STZ- induced diabetic rats. So the plant is considered as one of the possible sources for the isolation of new oral antihypoglycemic agents.

Conclusion

It is concluded that antimicrobial activity of black pepper evaluated the antibacterial potential of aqueous decoction of *Piper nigrum* L. (black pepper), against different bacterial isolates from oral cavity of individual volunteers.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

References:

- Mathew PJ.; Mathew PM.; Kumar V. Graph clustering of *Piper nigrum* L. (black pepper). *Euphytica*. 2001, 18: 257-264.
- Srinivasan. Black pepper and its pungent principle piperine: a review of diverse physiological effects. *Crit Rev Food Sci Nutr*. 2007, 47(8): 735-748.
- Singh VK.; Singh P.; Mishra A.; Patel A.; Yadav KM. Piperine: delightful surprise to the biological world, made by plant “pepper” and a great bioavailability enhancer for our drugs and great bioavailability enhancer for our drugs and supplements. *World J Pharmac Res*. 2014, 3(6): 2084-2098.
- Awen BZ.; Ganapati S.; Chandu BR. Influence of *Sapindus mukorossi* on the permeability of ethyl cellulose free film for transe dermal use. *Res J Pharma Biol Chem Sci*. 2010, 1: 35-38.
- Hussain A.; Naz S.; Nazir H.; Shinwari ZK. Tissue culture of black pepper (*Piper nigrum* L.) in Pakistan. *Pak J Bot*. 2011, 43: 1069-1078.
- Ahmad N.; Fazal H.; Abbasi BH.; Farooq S.; Ali M.; Khan MA. Biological role of *Piper nigrum* L. (black pepper): a review. *Asian Pac J Trop Biomed*. 2015, S1945-S1953.
- .Sujatha R.; Luckin CB.; Nazeem PA. Histology of organogenesis from callus cultures of black pepper (*Piper nigrum* L). *J Trop Agric*. 2003, 41: 16-19.
- Parganiha R.; Verma S.; Chandrakar S.; Pal S, Sawarkar HA.; Kashyap P. In vitro anti-asthmatic activity of fruit extract of *Piper nigrum* (Piperaceae). *Int J Herbal Drug Res*. 2011, 1: 15-18.
- Fan LS.; Muhmad R.; Omar D.; Rahimani M. Insecticidal properties of *Piper nigrum* fruit extracts and essential oils against *Spodoptera litura*. *Int J Agric Biol*. 2011, 13: 517-522.

- Manoj P.; Soniya EV.; Banerjee NS. Recent studies on well know spice Piper longum Linn. *Natural Product Radiance*. 2004, 3(4):222-227
- Sumy O.; Ved DK.; Krishnan R. Tropical Indian Medicinal Plants, Propagation Methods, 2000, 268-269
- Banerjee NS.; Manoj P.; Das MR. Malesex associated RAPD markers in Piper longum Linn. *Curr Sci*, 1999, 77(5), 693- 695.
- Viswanathan TV. Medicinal and Aromatic Plants, KL Chadha and R Gupta (eds), Malhotra Publishing House, New Delhi, 1995, 373-383.
- Satyavati G.; Gupta AK.; Tondon N. *Medicinal plants of India*, 1987, Vol. 2, Indian Council of Medicinal research, New Delhi
- Parmar VS.; Jain SC.; Bisht KS.; Jain R.; Taneja P.; Jha A.; Tyagi OD. Phytochemistry of the genus Piper. *Phytochemistry* 1997, 46: 597-673.
- Fan LS.; Muhmad R.; Omar D, Rahimani M. Insecticidal Properties of Piper nigrum Fruit Extracts and Essential Oils against Spodoptera litura. *Inter. J. Agric. Biol* 2011; 13: 517-522.
- Wattanathorn J.; Chonpathompikunlert P.; Muchimapura S.; Priprem A.; Tankamnerdthai O. Piperine, the potential functional food for mood and cognitive disorders. *Food Chem Tech* 2008, 46: 3106-3110.
- Bhardwaj RK.; Glaeser H.; Becquemont L.;Klotz U.; Gupta SK.; Fromm MF. Piperine, a major constituent of Black pepper, inhibits human P-glycoprotein and CYP3A4. *J. Pharma Exp Therapeutics* 2007, 302: 645-650.
- Cotinguiba F.; Manke K.; Furlan M.; Vogt T. Molecular investigations of Piper nigrum (Black pepper) fruits in search for natural products biosynthetic target genes. *Congresso Brasileiro de Genetica*, 2011, 30: 16.
- Wei K.; Li W.; Koike K.; Pei Y.; Chen Y.; Nikaido T. New amide alkaloids from the roots of Piper nigrum. *J. Nat. Prod* 2004, 67: 1005-1009.
- Molteni R.; Calabrese F.; Bedogni F.;Tongiorgi E.; Fumagalli F.; Racagni G.; Riva MA. Chronic treatment with fluoxetine upregulates cellular BDNF mRNA expression in rat dopaminergic regions. *Inter J. Neuropsychopharma* , 2006, 9: 307-317.
- Lee CS.; Han ES.; Kim YK. Piperine inhibition of 1-methyl-4- phenylpyridinium-induced mitochondrial dysfunction and cell death in PC12 cells. *Europ. J. Pharma* 2006, 537: 37-44.

Lee SA.; Hong SS.; Han XH.; Hwang JS.; Oh GJ.; Lee KS.; Lee MK.; Hwang BY.; Ro JS. Piperine from the fruits of *Piper longum* with inhibitory effect on monoamine oxidase and antidepressant-like activity. *Chem. Pharma. Bull* 2005, 53: 832-835.

Kumar A.; Khan IA.; Koul S, Koul JL.; Taneja SC.; Ali I.; Ali F.; Sharma S.; Mirza ZM.; Kumar M.; Sangwan PL.; Gupta P.; Thota N.; Qazi GN. Novel structural analogues of piperine as inhibitors of the NorA efflux pump of *Staphylococcus aureus*. *J. Antimicrob Chemother* 2008, 61: 1270-1276.

Chonpathompikunlert P.; Wattanathorn J.; Muchimapura S. Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease. *Food. Chem. Toxicol* 2010 ,48: 798-802.

Ramji MT, Deepthi K, Lakshmi KA, Uma devi P. In silico docking analysis of piperine amino acid analogues against carcinogenic activating enzymes. *Biotechnology* 2011; doi:10.4172/jpb.1000240.

Kolhe SR.; Borole P.; Patel U. Extraction and evaluation of piperine from *Piper nigrum* linn. *Inter. J. Appl. Biol. Pharma.l Tech* 2011, 2: 144-149.

Kokate CK.; Purohit AP.; Gokhale SB. Pharmacognosy, 42nd Edition, Nirali Prakashan, 2008, Page no. 11.56-11.58.

Mann A.; Biopotency role of culinary spices and herbs and their chemical constituents in health and commonly used spices in Nigerian dishes and snacks. *African J Food Sci* 2011, 5: 111-124.

Ahmad N.; Fazal H.; Ahmad I.; Abbasi BH. Free Radical Scavenging (DPPH) Potential in Nine Mentha Species. *Journal of Toxicology and Industrial Health* 2011, Doi. 10.1177/0748233711407238

Vijaykum RS.; Surya D.; Nalini N. Antioxidant efficacy of black pepper(*piper nigum*. L) and piperine in rats with high fat diet induced oxidative stress, *Redox Rep* , 2002, 9;105-110

Selvendiran K.; sakhisekaran D. Chemo preventive effect of piperine on modulating lipid peroxidation and membrane bound enzymes in benzo(a) pyrene induced lung carcinogenesis, *Biomed. Pharmacother* 2004, 58; 264-267

Ahmed N.; Fazal H.; Abbasi BH.; Rashid M, Mahmood. T Fatima N efficient regeneration and antioxidant potential in regenerated tissues of piper nigrum L plant cell, tissue and organ culture, *Pharma Res*, 2010, 102: 29-134

Hritcu L.; Noumedem JA.; Cioanea O.; Hancianu, M.; Kuete V. Methanol extract of piper nigrum fruits improves memory impairment by decreasing brain oxidative stress in amyloid beta(1-42) rat model of Alzheimer's diseases. *Cell mol Neurobiol* , 2014, 34: 437-449

Damanhoury ZA.; and Ahmad A. A review on therapeutic potential of Piper nigrum L. (Black Pepper): The King of Spices. *Medicinal and Aromatic Plants*. 2014, 3(3): 1-6.

Selvendiran K.; Sakthisekaran D. Chemoprotective effect of piperine on modulating lipid peroxidation and membrane bound enzymes in benzo (a) pyrene induced lung carcinogenesis. *Biomedicine and Pharmacotherapy*. 2004, 58: 264-267.

Mona AM.; Abo Z and Ayman A. The anti-mutagenic activity of piperine against mitomycin C induced sister chromatid exchange and chromosomal aberration in mice. *Nature and Sci*. 2009, 7: 72-78

Khan M.; Siddiqui M. Antimicrobial activity of Piper fruits. *Nat prod Rad*, 2007, 611-116

Reddy Srinivas P.; Kaiser Jamil.; Madhusudhana P.; Anjali G and Das B. Antibacterial activity of isolates from Piper longum and Taxus baccata, *Pharma Biol*, 2001, 39(3) 236-238

Vijayakumar RS.; Antioxidant efficacy of black pepper (Piper nigrum L.) and piperine in rats with high fat diet induced oxidative stress. *Redox Rep* 2004, 9: 105-110.

Singh A.; Duggal S. Piperine- Review of Advances in Pharmacology. *Inter. J. Pharma. Sci. Nanotech* 2009, 2: 615-620.

Gurumurthy P.; Vijayalatha S.; Sumathy A .Hepatoprotective effect of aqueous extract of Piper longum and piperine when administered with antitubercular drugs. *The Bioscan*. 2012, 7(4): 661-663

Matsuda H.; Ninomiya K.; Morikawa T.; Yasuda D.; Yamaguchi I.; Yoshikawa M. Protective effects of amide constituents from the fruit of Piper chaba on D- galactosamine/TNF-alpha induced cell death in mouse hepatocytes. *Bioorg Med Chem Lett* , 2008, 18: 2038-2042

Sabinsa Corporation. Sabinasa corporation home page. East Windsor, NJ: Sabinsa Corporation. 2011; Available from: www.sabinsa.com.

Badmaev V.; Majeed M.; Norkus EP. Piperine, an alkaloid derived from black pepper, increases serum response of beta-carotene during 14 Days of oral beta-carotene supplementation. *Nutr Res.* 1999; 19: 381-388.

Majeed M.; Prakash LTHP. An all natural delivery system adjuvant. In delivery system handbook for personal care and cosmetic products: Technology, applications and formulations. Meyer RR, ed, *William and Andrew Publishing*, 2005.

Garg SK.; Anti-fertility effects of Embelia ribes and Piper longum in female albino rats. *Fitoterapia.* 1981, 52(4):167- 169

Munshi SR.; Ljungkvist I. Antifertility activity of an indigenous plant preparation (ROC-101). 3. Effect on ultrastructure of the rat uterine luminal epithelium. *Ind. J. Med. Res.* 1972, 60(12) 1791-1793.

Mao QQ.; Huang Z2.; Zhong XM2.; Xian YF3. Ip SP4 Piperine reverses the effects of corticosterone on behaviour and hippocampal BDNF expression in mice. *Neuro chem Int* 2014, 74: 36-41.

Nabi SA.; Kasetti RB.; Sirasanagandla S. Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. *BMC Complementary and Alternative Medicine.* 2013, 13: 37

Patel, M., Patel, D., Shah, U., Patel, A., Solanki, N., Patel, S., Patel, S. and Patel, B., 2022. Piperine: Medicinal, Analytical and Therapeutics Perspective. *Current Bioactive Compounds*, 18(1), pp.3-23.

Umang Shah and Ankita Jasani,. UV spectrophotometric and RP-HPLC methods for simultaneous estimation of isoniazid, rifampicin and piperine in pharmaceutical dosage form. *Int J Pharm Pharm Sci*, 2014 6(10), pp.274-80.

Vyas, N., Gamit, K., Khan, M.Y., Panchal, S. and Pundarikakshudu, K., 2011. Simultaneous estimation of curcumin and piperine in their crude powder mixture and ayurvedic formulation using high performance thin layer chromatography. *Int J Res Pharm Biomed Sci*, 2(01), pp.231-6.

Zaveri, M., Khandhar, A., Patel, S. and Patel, A., 2010. Chemistry and pharmacology of Piper longum L. *International journal of pharmaceutical sciences review and research*, 5(1), pp.67-76.

UNDER PEER REVIEW